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**A choice that's right**





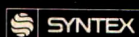
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- ▼ 29% lower progestin dose than 1/35<sup>1</sup>
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- ▼ Supplied in the patient-preferred Walleto™ pill dispenser.<sup>4</sup>

Serious as well as minor side effects have been reported following the use of all oral contraceptives. These include thromboembolic disease. Please see brief summary of full prescribing information on next page.

1. 1/35 formulations contain 1.0 mg norethindrone with 0.035 mg ethinyl estradiol. BTB comparisons are based on the first three cycles of use. Data available from Syntex Laboratories, Inc.
2. Wynn V, Niththyananthan R: The effect of progestins in combined oral contraceptives on serum lipids with special reference to high-density lipoproteins. *Am J Obstet Gynecol* 142:766-772, 1982.
3. Wynn V: Effect of duration of low-dose oral contraceptive administration on carbohydrate metabolism. *Am J Obstet Gynecol* 142:739-746, 1982.
4. In an independent survey the Walleto™ pill dispenser was preferred to the Ortho Dialpak by 7 out of 10 prospective OC patients. Data available from Syntex Laboratories, Inc.



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# TRI - NORINYL<sup>®</sup>

(NORETHINDRONE AND ETHINYL ESTRADIOL)

**Because your common goal is confidence.**



**TRI-NORINYL® 21-Day Tablets** (each blue tablet contains norethindrone 0.5 mg with ethinyl estradiol 0.035 mg and each green tablet contains norethindrone 10 mg with ethinyl estradiol 0.035 mg)

**TRI-NORINYL® 28-Day Tablets** (each blue tablet contains norethindrone 0.5 mg with ethinyl estradiol 0.035 mg, each green tablet contains norethindrone 10 mg with ethinyl estradiol 0.035 mg, and orange tablets are inert)

**BREVICON® 21-Day Tablets** (norethindrone 0.5 mg with ethinyl estradiol 0.035 mg)

**BREVICON® 28-Day Tablets** (21 norethindrone 0.5 mg with ethinyl estradiol 0.035 mg, tablets followed by 7 inert tablets)

**NORINYL® 1 + 35 21-Day Tablets** (norethindrone 1 mg, with ethinyl estradiol 0.035 mg)

**NORINYL® 1 + 35 28-Day Tablets** (21 norethindrone 1 mg, with ethinyl estradiol 0.035 mg, tablets followed by 7 inert tablets)

**NORINYL® 1 + 50 21-Day Tablets** (norethindrone 1 mg, with mestranol 0.05 mg)

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**NORINYL® 1 + 80 28-Day Tablets** (21 norethindrone 1 mg, with mestranol 0.08 mg, tablets followed by 7 inert tablets)

**NORINYL® 2 mg. Tablets** (norethindrone 2 mg, with mestranol 0.1 mg)

**NOR-Q.D.®** (norethindrone) Tablets 0.35 mg.

**Indications:** Prevention of pregnancy. **DOSE-RELATED RISK OF THROMBOEMBOLISM.** Because studies have shown a positive association between OC estrogen dose and risk of thromboembolism, it is prudent to minimize estrogen exposure. Prescribe an OC with the least amount of estrogen compatible with an acceptable pregnancy rate and patient acceptance. Start new users on OCs containing 0.05 mg or less of estrogen.

**Contraindications:** 1. Known or suspected pregnancy (see Warning #5). 2. Thrombophlebitis or thromboembolic disorders. 3. Past history of deep vein thrombophlebitis or thromboembolic disorders. 4. Undiagnosed abnormal genital bleeding. 5. OCs should not be used by women who have or have had any of the following: a cerebral vascular or coronary artery disease, including myocardial infarction, b. known or suspected carcinoma of the breast, c. known or suspected estrogen dependent neoplasia, d. benign or malignant liver tumor that developed during use of OCs or other estrogen containing products.

**WARNINGS:** Cigarette smoking increases the risk of serious cardiovascular side effects from OC use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use OCs should be strongly advised not to smoke.

The use of OCs is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, liver tumor, gall bladder disease, visual disturbances, fetal abnormalities, and hypertension. Practitioners prescribing OCs should be familiar with the following information relating to these risks.

1. **Thromboembolic Disorders and Other Vascular Problems:** An increased risk of thromboembolic and thrombotic disease associated with OC use is established. One study demonstrated an increased relative risk for fatal venous thromboembolism and several studies demonstrated it for non-fatal venous thromboembolism. They estimate that OC users are 4-11 times more likely than nonusers to develop these diseases without evident cause. One British study reported an excess death rate of 40% in OC users, most of which resulted from cardiovascular disease. Another British study showed a lower death rate in OC users than controls; an increase in cardiovascular deaths was seen but was not statistically significant. A U.S. prospective study failed to disclose increased mortality rates from thromboembolic disorders, but a subsequent analysis of a retrospective, case-control study showed significant increases in venous thromboembolism. **CEREBROVASCULAR DISORDERS:** Two American studies demonstrated an increased relative risk for stroke not shown in prior British studies. In an American study of cerebrovascular disorders in women with and without predisposing causes, relative risk of hemorrhagic stroke was estimated as 2.0 times greater and thrombotic stroke as 4-9.5 times greater in users than nonusers. A 5-fold increased risk of fatal infarction compared to nonusers of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preclampsia/torax) the higher the risk of developing MI, regardless of OC use. OCs were an additional risk factor. In terms of relative risk, it has been estimated that nonsmoking OC users (smoking is considered a major predisposing condition to MI) are twice as likely to have a fatal MI as nonsmoking nonusers. OC users who are smokers have a 5-fold increased risk of fatal infarction compared to nonsmoking users, and a 10-12-fold increased risk compared to nonsmoking nonusers. The number of cigarettes smoked is important. In determining the importance of these relative risks, baseline rates for various age groups must be considered. (Estimates are based on British vital statistics which show acute MI death rates 2-3 times less than in the U.S. so U.S. death rates could be higher.) Importance of other predisposing conditions in determining relative and absolute risks has not been quantified, other synergistic action may exist. **RISK OF DOSE:** Using data from several national adverse reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to estrogen dose in OCs. OCs with 0.1 mg or more of estrogen were associated with a higher risk of thromboembolism than those containing 0.05-0.08 mg but quantity of estrogen may not be the sole factor. This was supported by a U.S. study. A British study found a positive association between dose of progestogen or estrogen and certain thromboembolic conditions. Swedish authorities noted decreased reporting of thromboembolic episodes when higher estrogen preparations were no longer prescribed. Careful epidemiological studies to determine degree of thromboembolic disease risk associated with progestogen-only OCs have not been done. Thromboembolic disease has been reported in women using these products, and they should not be considered free of excess risk. **PERSISTENCE OF RISK:** Two studies have suggested an increased risk may persist for 6 years after discontinuation of OC use for cerebrovascular disease and 9 years for MI; another study suggested persistence of risk for subarachnoid hemorrhage. **ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES.** A large British prospective study estimated mortality rate per 100,000 women per year from circulatory system diseases for OC users and nonusers according to age, smoking habits, and duration of use. The overall annual excess death rate for OC users was estimated to be 20/100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000). Risk is concentrated in long-term users and in smokers, and may persist after OC discontinuation. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all occurred in women 35 or older. An update provided the following rates: ages 15-34—1/1000 for nonsmokers and 1/2000 for smokers; ages 45 and over—1/2500 for nonsmokers and 1/500 for smokers. Risk appeared to increase with parity, but not with duration of use. Until more women under 35 with continuous use for 5 or more years are available, it is not possible to assess relative risk for this age group. Data from a variety of sources have been analyzed to estimate risk of death associated with various methods of contraception. Estimates include combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease for OCs) plus risk attributable to pregnancy or abortion if the method fails (which varies with the effectiveness of the contraceptive method). Data are shown in Table 1 below. The study concluded that mortality associated with all contraceptive methods is below that of childbirth, except for OCs in women over 40 who smoke. (Rates given for pill only/smokers for each age are for smokers as a class. For "heavy" smokers (more than 15 cigarettes a day), rates would be about double, for "light" smokers (less than 15), about half.) The lowest

mortality is with the condom or diaphragm backed up by early abortion. The study also concluded that OC users who smoke, especially over 30, have greater mortality risk than OC users who do not smoke.

**Table 1. Risk of thromboembolic and thrombotic disease associated with OCs increases with age after 30 and, for MI, is further increased by hypertension, hyperlipidemia, obesity, diabetes, or history of preclampsia/torax, and especially by smoking. The following chart gives a gross estimate of risk of death from circulatory disorders associated with OC use.**

SMOKING HABITS AND OTHER PREDISPOSING CONDITIONS—RISK ASSOCIATED WITH USE OF OCs				
Age	Below 30	30-39	40+	
Heavy smokers	A	B	A	
Light smokers	D	C	B	
Nonsmokers	D	C	B	
(no predisposing conditions)	D	C,D	C	
Nonsmoker	D	C,B	B,A	
(other predisposing conditions)				

A—Use associated with very high risk.

B—Use associated with high risk.

C—Use associated with moderate risk.

D—Use associated with low risk.

Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, discontinue OC immediately. A 4-6 fold increased risk of post-surgery thromboembolic complications has been reported in OC users. If feasible, discontinue OCs at least 4 weeks before surgery associated with increased risk of thromboembolism or prolonged immobilization. Before surgery, OC user after major surgery or bedrest, balance risks of post-surgery thromboembolic complications with contraceptive needs. Data suggest varicose veins substantially increase risk of superficial venous thrombosis of the leg, the risk depending on severity of the varicosities. 2. **Ocular Lesions:** Neuro-ocular lesions such as optic neuritis or retinal thrombosis have been associated with OC use. Discontinue OC if there is unexplained, sudden or gradual, partial or complete loss of vision, onset of proptosis or diplopia, papilledema, or retinal vascular lesions, and institute appropriate diagnostic and therapeutic measures. 3. **Carcinoma:** Long-term continuous administration of natural or synthetic estrogen in certain animals increases certain tumors, benign or malignant, such as breast, cervix, vagina, uterus, ovary, pituitary and liver. Certain synthetic progestogens, none currently in OCs, increase the incidence of mammary nodules, benign and malignant, in dogs. Several retrospective case-control studies reported an increased relative risk (3.1-13.3 times) associating endometrial carcinoma with prolonged use of estrogens in postmenopausal women. One publication reported the first 30 cases submitted to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the adenocarcinomas found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding when OCs were first given, polycystic ovaries), nearly all occurred in women who had used sequential OCs, which are no longer marketed. No statistical association has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs, although individual cases have been reported. Studies have shown no increased risk of breast cancer to OC or estrogen users. One study found no overall increased risk of breast cancer in OC users but a greater risk was suggested for OC users with documented benign breast disease and for long-term (2-4 years) users. Another study found a history of breast cancer among grandmothers or aunts was significantly more frequent among breast cancer patients who had used an OC continuously for one or more years than among nonusers with breast cancer. One other study indicated an increasing risk of breast cancer in women taking menopausal estrogens, which increased with duration of follow-up. One author suggests that extended (over 6 years) OC use prior to first full-term pregnancy was associated with a significant relative increase in breast cancer. A case-control study of benign breast tumors in OC users has been well documented. One study reported malignant melanoma more frequently in OC users than controls and suggests an increased incidence of urinary tract and thyroid cancers. A prospective study of women with cervical dysplasia found an increase in severity and conversion to cancer in *situ* in OC users compared with nonusers. This became statistically significant after 4-5 years of use. Nonreversal of dysplasia within the first 6 months of pill use was suggested to predict progression after prolonged exposure. One study disclosed an increased risk of cancer of the cervix (largely carcinoma *in situ*) in OC users under 40, particularly those who had used OCs over 4 years. There have been other reports of microglandular hyperplasia of the cervix in OC users. One study reported an association between OC use and endocervical adenocarcinoma. In summary, there is no confirmed evidence from human studies of increased risk of cancer associated with OCs. Close clinical surveillance of all OC users is nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, take appropriate diagnostic measures to rule out malignancy. Monitor OC users with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms with particular care. 4. **Liver Tumors:** Sudden severe abdominal pain or shock may be due to rupture and hemorrhage of a liver tumor. There have been reports associating benign or malignant liver tumors with short-term and long-term OC use. One study reported use of OCs with high hormonal potency and age over 30 may further increase risk of hepatocellular adenoma. Two studies relate risk with duration of use, risk being much greater after 4 or more years of use. Long-term OC users have an estimated annual incidence of hepatocellular adenoma of 3-4/100,000. Although an uncommon lesion, it should be considered in women presenting with an "acute abdomen." The tumor may cause serious complications. 5. **Use in or Immediately Preceding Pregnancy, Birth Control, Offspring, and Malignancy in Female Offspring:** Use of female sex hormones—estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. Females exposed *in utero* to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1/1000 exposures or less. Although there is no evidence that OCs further enhance the risk of developing this type of malignancy, such OC users should be monitored with particular care. A high percentage of women exposed to diethylstilbestrol (30-90%) have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are a precursor of vaginal malignancy. Male children so exposed may develop urogenital tract abnormalities. Although similar data are not available on other estrogens, it cannot be presumed that they would not induce similar changes. Increased risk of congenital anomalies, including heart and limb defects, has been reported following use of sex hormones, including OCs, in pregnancy. One case-control study estimated a 4.7-fold increased relative risk of limb-reduction defects in infants exposed *in utero* to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some exposures involved only a few days of treatment. Data suggest risk of limb-reduction defects in exposed fetuses is somewhat less than 1/1000 live births. In a large prospective study, cardiovascular defects in children born to women who received female hormones, including OCs, during early pregnancy occurred at 18-21,000 births, compared to 7.8/1000 for children not so exposed *in utero*. These results are statistically significant. A Welsh study found a statistically significant excess of renal and urogenital malformations in children of OC users (within 3 months) than among controls. The incidence of twin births may be increased for women who conceive shortly after discontinuing OC use. In the past, female sex hormones were used during pregnancy in an attempt to treat threatened or habitual abortion. There is evidence that estrogens are ineffective, and there is no evidence from well controlled studies that progestogens are effective for these uses. There is some evidence that the triphasic and quadruphasic types of OCs, which are increasing among abortifacients from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. If the patient has not adhered to the prescribed schedule, consider possible pregnancy at the first missed period (or 45 days from the last menstrual period if progestogen-only OCs are used) and discontinue OC use until pregnancy has been ruled out. For any patient who has missed two consecutive periods, rule

out pregnancy before continuing the OC. If pregnancy is confirmed, tell the patient about potential risks to the fetus and discuss advisability of continuing the pregnancy. Women who discontinue OCs to become pregnant should use an alternate form of contraception for a period of time before attempting to conceive. A 3-month period is supported by a study suggesting increased frequency of neural tube defects in women impregnated during the first 3 months after cessation of OC use. Do not use progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding as a pregnancy test. 6. **Gall Bladder Disease:** Studies report increased risk of gall bladder disease in OC users. In one study, increased risk appeared after 2 years of use and doubled after 4-5 years. In another study, an increased risk was apparent between 6 and 12 months. 7. **Carbohydrate and Lipid Metabolic Effects:** Because a decrease in glucose tolerance has been observed in a significant percentage of patients on OCs, prediabetic and diabetic OC users should be carefully observed. An increase in triglycerides and total phospholipids has been observed in OC users but its clinical significance is unknown. 8. **Elevated Blood Pressure:** An increase in blood pressure has been reported with OC use. Hypertension may occur within a few months of beginning OCs. In the first year of use, incidence of hypertension may be no higher in OC users than in nonusers. Incidence in users increases with exposure and in the fifth year of use is 2.5-3 times that in the first year. Age is strongly correlated with hypertension in OC users. Women with a history of elevated blood pressure (hypertension), preexisting renal disease, history of toxemia or elevated blood pressure during pregnancy, familial tendency to hypertension or its consequences, or history of excessive weight gain or fluid retention during the menstrual cycle may be more likely to develop elevated blood pressure when given OCs and should be monitored closely. Even though elevated blood pressure may remain within the "normal" range, closely watch elevations, particularly for women with other risk factors for cardiovascular disease or stroke. High blood pressure may or may not persist after OC discontinuation. 9. **Headache:** Discontinue OC and evaluate the cause if onset or exacerbation of migraine or development of a new pattern of headache which is recurrent, persistent, or severe. 10. **Bleeding Irregularities:** Breakthrough bleeding, spotting, and missed menses often make users discontinue OCs. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, consider nonfunctional causes. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, use adequate diagnostic measures to rule out pregnancy or malignancy. If pathology has been excluded, time or another formulation may solve the problem. While potentially useful in minimizing menstrual irregularity, change to an OC with a higher estrogen content only if necessary since this may increase risk of thromboembolic disease. Women with a history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may tend to remain anovulatory or to become amenorrheic after OC discontinuation. Women with these preceding problems should be advised of this and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may occur in women without previous irregularities. A higher incidence of galactorrhea and of pituitary tumors (e.g., adenomas) has been associated with amenorrhea in former users compared with nonusers. One study reported a 16-fold increased incidence of pituitary prolactin-secreting tumors among patients with postpill amenorrhea when galactorrhea was present. 11. **Fertility:** There is evidence of impairment of fertility in women discontinuing OCs in comparison with other contraceptive methods, which appears to be independent of duration of use. While impairment diminishes with time, there is an appreciable difference in results in nulliparous women for OC and other groups 30 months after discontinuation of birth control. For parous women the difference is not apparent 30 months after cessation of contraception. 12. **Ectopic Pregnancy:** Ectopic as well as intrauterine pregnancy may occur in OC failures. In progestogen-only OC failures, the ratio of ectopic to intrauterine pregnancies is higher than in nonusers, since the drugs are more effective in preventing intrauterine than ectopic pregnancies. 13. **Breast Feeding:** OCs in the postpartum period may interfere with lactation by decreasing quantity and quality of breast milk. A small fraction of OC hormonal agents has been identified in milk of mothers receiving OCs. Effects, if any, on the breast-fed child have not been determined. 14. **Other Considerations:** Use OC until the time of delivery. **Precautions:** GENERAL 1. Take a complete medical and family history before starting OCs. Pretreatment and periodic physical exams should include special studies to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant lab tests. As a general rule, OCs should not be prescribed for longer than 1 year without another physical. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may be affected. The clinical significance is unknown. 3. Serum folate levels may be depressed by OC use. Since the pregnant woman is predisposed to folate deficiency and incidence of folate deficiency increases with increasing gestation, if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and related complications. 9. Advise the pathologist of OC use when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing OCs. For example, a decreased sulfobromophthalen retention, b. increased prothrombin and factors VII, VIII, IX and X, decreased antithrombin 3; increased norepinephrine-induced platelet aggregation, c. increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered, d. decreased androgen excretion, e. Reduced response to pituitary gonadotropin, f. Increased phospholipids and triglycerides, g. Temporally decreased glucose tolerance. 11. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist and temporary or permanent cessation of wear considered. **DRUG INTERACTIONS:** OCs may be less effective and there may be increased breakthrough bleeding because of interactions with rifampin, isoniazid, ampicillin, tetracycline, neomycin, penicillin V, chloramphenicol, sulfonamides, nitrofurantoin, barbiturates, phenytoin, primidone, analgesics, tranquilizers, anti-migraine preparations, and antihistamines. OCs may alter effectiveness of such other drugs as anti-coagulants, anticonvulsants, tricyclic anti-depressants, anti-hypertensive agents (e.g., guanethidine), vitamins, hypoglycemic agents, tranquilizers, hypnotic preparations, and theophylline. **CARCINOGENESIS:** See Warnings. **PREGNANCY:** Pregnancy category X. See Contraindications and Warnings. **NURSING MOTHERS:** See Contraindications and Warnings. **Adverse Reactions:** An increased risk of the following serious adverse reactions has been associated with OC use (see Warnings): thrombophlebitis, thrombosis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, mesenteric thrombosis, liver tumors, cerebral hemorrhage, hypertension, gall bladder disease, congenital anomalies, neuro-ocular lesions, retinal thrombosis and an optic neuritis, Raynaud's disease, arterial thromboembolism. The following adverse reactions have been reported in OC users and are believed to be drug related: bleeding irregularities (breakthrough bleeding, spotting, missed menses during treatment, amenorrhea after treatment), gastrointestinal symptoms (nausea, vomiting, bloating, abdominal cramps), dysmenorrhea, infertility after discontinuance of treatment, edema, chloasma or melasma which may persist when drug is discontinued, breast changes (tenderness, enlargement, and secretion), intolerance to contact lenses, change in corneal curvature (steepening), change in weight (increase or decrease), change in cervical erosion and cervical secretion, possible diminution in lactation when given immediately postpartum, cholestatic jaundice, migraine, increase in size of uterine leiomyomata, rash (allergic), mental depression, reduced tolerance to carbohydrates, increased postpill amenorrhea, prolactin-secreting pituitary tumors, chills. The following adverse reactions have been reported in OC users and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, impaired renal function, malignant nephroses (hemolytic uremic syndrome). **Information for the Patient** (See Patient Package Insert).

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Palo Alto, California 94304





# An established standard of efficacy

in serious pelvic infection

- Clinically effective against *Bacteroides fragilis* and other anaerobes commonly found in polymicrobial infections.
- Clinically effective against many gram-positive aerobes (eg, *Staphylococcus aureus*, group B streptococci) encountered in polymicrobial infections.

Clindamycin has been associated with *Clostridium difficile* colitis as have many other antibiotics (eg, cephalosporins, penicillins, and ampicillin). See Warnings in summary of prescribing information on the adjacent page.

**Cleocin  
Phosphate**<sup>®</sup> STERILE  
SOLUTION  
(clindamycin phosphate injection)

900 mg q8h

Postcesarean endomyometritis  
(artist's interpretation)

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of Caring  
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CLEOCIN PHOSPHATE® Sterile Solution  
CLEOCIN HCl® Capsules  
(clindamycin)

#### WARNING

Clindamycin therapy has been associated with severe colitis which may end fatally. Therefore, it should be reserved for serious infections where less toxic antimicrobial agents are inappropriate, as described in the Indications Section. It should not be used in patients with nonbacterial infections, such as most upper respiratory tract infections. Studies indicate a toxin(s) produced by *Clostridia* is one primary cause of antibiotic associated colitis. Cholestyramine and colestipol resins have been shown to bind the toxin *in vitro*. See WARNINGS section. The colitis is usually characterized by severe, persistent diarrhea and severe abdominal cramps and may be associated with the passage of blood and mucus. Endoscopic examination may reveal pseudomembranous colitis.

When significant diarrhea occurs, the drug should be discontinued or, if necessary, continued only with close observation of the patient. Large bowel endoscopy has been recommended.

Antiperistaltic agents such as opiates and diphenoxylate with atropine (Lomotil) may prolong and/or worsen the condition. Vancomycin has been found to be effective in the treatment of antibiotic associated pseudomembranous colitis produced by *Clostridium difficile*. The usual adult dose is 500 milligrams to 2 grams of vancomycin orally per day in three to four divided doses administered for 7 to 10 days. Cholestyramine or colestipol resins bind vancomycin *in vitro*. If both a resin and vancomycin are to be administered concurrently, it may be advisable to separate the time of administration of each drug.

Diarrhea, colitis, and pseudomembranous colitis have been observed to begin up to several weeks following cessation of therapy with clindamycin.

#### INDICATIONS

Serious infections caused by susceptible anaerobic bacteria. Patients with serious infections due to susceptible strains of streptococci, pneumococci, and staphylococci in whom its use should be reserved for penicillin-allergic patients or other patients for whom, in the judgment of the physician, a penicillin is inappropriate.

Consider the nature of the infection and the suitability of less toxic alternatives (e.g., erythro-

mycin). Bacteriologic studies should be performed to determine the causative organisms and their susceptibility to clindamycin.

#### CONTRAINDICATIONS

History of hypersensitivity to clindamycin or lincomycin.

#### WARNINGS

See WARNING box. A toxin produced by *Clostridia* is one primary cause of antibiotic associated colitis. Cholestyramine and colestipol resins have been shown to bind the toxin *in vitro*. Mild cases of colitis may respond to drug discontinuance alone. Moderate to severe cases should be managed promptly with fluid, electrolyte and protein supplementation as indicated. Vancomycin has been found to be effective in the treatment of antibiotic associated pseudomembranous colitis produced by *Clostridium difficile*. The usual adult dosage is 500 mg to 2 grams of vancomycin orally per day in 3 or 4 divided doses for 7 to 10 days. Systemic corticoids and corticoid retention enemas may help relieve the colitis. Other causes of colitis should also be considered.

A careful inquiry should be made concerning previous sensitivities to drugs and other allergens. Because antagonism has been demonstrated between clindamycin and erythromycin *in vitro*, these drugs should not be administered concurrently. *Usage in Pregnancy*: Safety has not been established. *Usage in Newborns and Infants*: Appropriate monitoring of organ system functions is desirable. *Nursing Mothers*: Clindamycin has been reported to appear in breast milk in ranges of 0.7 to 3.8 mcg/ml. *Usage in Meningitis*: Since clindamycin does not diffuse adequately into the cerebrospinal fluid, it should not be used to treat meningitis.

**SERIOUS ANAPHYLACTOID REACTIONS REQUIRE IMMEDIATE EMERGENCY TREATMENT WITH EPINEPHRINE. OXYGEN AND INTRAVENOUS CORTICOSTEROIDS SHOULD ALSO BE ADMINISTERED AS INDICATED.**

#### PRECAUTIONS

Older patients with associated severe illness may tolerate diarrhea less well. When clindamycin is indicated in these patients, they should be carefully monitored for change in bowel frequency. Prescribe with caution in individuals with a history of gastrointestinal disease, particularly colitis and also in atopic individuals. Indicated surgical procedures should be performed in conjunction with therapy. Patients with severe renal disease and/or very severe hepatic disease accompanied by severe metabolic aberrations should be dosed with caution and serum clindamycin levels monitored during high dose therapy.

During prolonged therapy, periodic liver and kidney function tests and blood counts should be

performed. Use may result in overgrowth of non-susceptible organisms, particularly yeasts. Clindamycin has neuromuscular blocking properties and may enhance other neuromuscular blocking agents. Use with caution in patients receiving such agents. Do not inject clindamycin IV undiluted as a bolus. Dilute prior to IV administration to 300 mg per 50 ml or more of diluent. Infuse over at least 10-60 minutes. (See Dosage and Administration.) CLEOCIN HCl Capsules contain FD&C Yellow No. 5 (tartrazine) which may cause allergic-type reactions (including bronchial asthma) in certain susceptible individuals, especially in patients who also have aspirin hypersensitivity.

#### ADVERSE REACTIONS

**Gastrointestinal**: Abdominal pain, nausea, vomiting and diarrhea. (See WARNING box.)

**Hypersensitivity Reactions**: Maculopapular rash and urticaria. Generalized mild to moderate morbilliform-like skin rashes are the most frequent adverse reactions. Rare instances of erythema multiforme, some resembling Stevens-Johnson syndrome, have been reported. A few cases of anaphylactoid reactions have been reported. If a hypersensitivity reaction occurs, the drug should be discontinued. The usual agents should be available for emergency treatment. **Liver**: Jaundice and abnormalities in liver function tests have been observed. **Hematopoietic**: Neutropenia, eosinophilia, agranulocytosis and thrombocytopenia have been reported; no direct etiologic relationship to concurrent clindamycin therapy has been made. **Local Reactions**: Pain, induration and sterile abscess have been reported after intramuscular injection and thrombophlebitis after intravenous infusion. Reactions can be minimized or avoided by giving deep intramuscular injections and avoiding prolonged use of indwelling intravenous catheters. **Musculoskeletal**: Rare instances of polyarthritides have been reported. **Cardiovascular**: Rare instances of cardiopulmonary arrest and hypotension have been reported following too rapid IV infusion. (See Dosage and Administration.) **Renal**: Renal dysfunction has rarely been observed. No direct relationship has been established.

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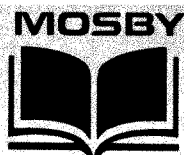


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For vulvovaginal candidiasis...

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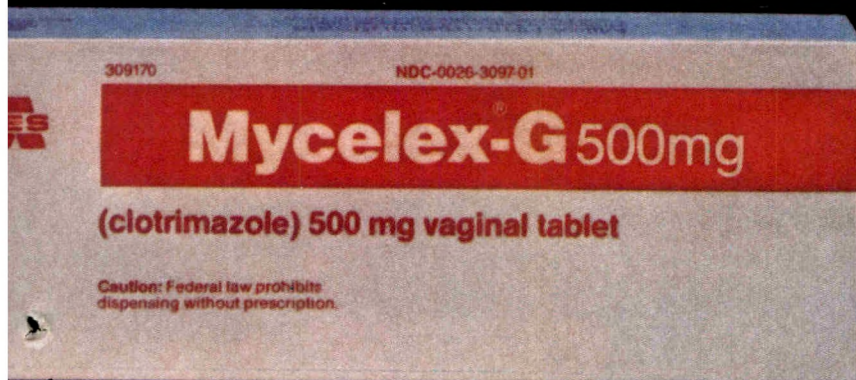
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**CONTRAINDICATIONS:** Mycelex-G 500 mg Vaginal Tablets are contraindicated in women who have shown hypersensitivity to any components of the preparation.

**WARNINGS:** None.

**PRECAUTIONS:** If there is a lack of response to Mycelex-G 500 mg Vaginal Tablets, appropriate microbiological studies should be repeated to confirm the diagnosis and rule out other pathogens before instituting another course of antimycotic therapy.

**CARCINOGENESIS:** No long term studies in animals have been performed to evaluate the carcinogenic potential of Mycelex-G 500 mg Vaginal Tablets intravaginally. A long term study in rats (Wistar strains) where clotrimazole was administered orally provided no indication of carcinogenicity.

**USAGE IN PREGNANCY: PREGNANCY CATEGORY B:** The disposition of <sup>14</sup>C-clotrimazole has been studied in humans and animals. Clotrimazole is poorly absorbed following intravaginal administration to humans, whereas it is rather well absorbed after oral administration.

In clinical trials, use of vaginally applied clotrimazole in pregnant women in their second and third trimesters has not been associated with ill effects. There are, however, no adequate and well-controlled studies in pregnant women during the first trimester of pregnancy.

Studies in pregnant rats given repeated intravaginal doses up to 100 mg/kg/day have revealed no evidence of harm to the fetus due to clotrimazole.

Repeated high oral doses of clotrimazole in rats and mice ranging from 50 to 120 mg/kg resulted in embryotoxicity (possibly secondary to maternal toxicity), impairment of mating, decreased litter size and number of viable young and decreased pup survival to weaning. However, clotrimazole was not teratogenic in mice, rabbits and rats at oral doses up to 200, 180 and 100 mg/kg, respectively. Oral absorption in the rat amounts to approximately 90% of the administered dose.

Because animal reproduction studies are not always predictive of human response, this drug should be used only if clearly indicated during the first trimester of pregnancy.

**ADVERSE REACTIONS:** Of 297 patients in double-blind studies with the 500 mg vaginal tablet, 3 of 149 patients treated with active drug and 3 of 148 patients treated with placebo reported complaints during therapy that were possibly drug related. In the active drug group, vomiting occurred in one patient, vaginal soreness with coitus in another, and complaints of vaginal irritation, itching, burning and dyspareunia in the third patient. In the placebo group, clitoral irritation occurred in one patient and dysuria, described as remotely related to drug, in the other. A third patient in the placebo group developed bacterial vaginitis which the investigator classed as possibly related to drug.

Eighteen (1.6%) of the 1116 patients treated with Mycelex-G in other formulations in double-blind studies reported complaints during therapy that were possibly drug-related. Mild burning occurred in six patients while other complaints such as skin rash, itching, vulvar irritation, lower abdominal cramps and bloating, slight cramping, slight urinary frequency, and burning or irritation in the sexual partner, occurred rarely.

**OVERDOSAGE:** No data available.

**DRUG ABUSE AND DEPENDENCE:** Drug abuse and dependence with Mycelex-G 500 mg Vaginal Tablets has not been reported.

**DOSAGE AND ADMINISTRATION:** The recommended dose is one tablet inserted intravaginally one time only, preferably at bedtime. In the event of treatment failure, that is, persistence of signs and symptoms of vaginitis after five days, other pathogens commonly responsible for vaginitis should be ruled out before instituting another course of antimycotic therapy.

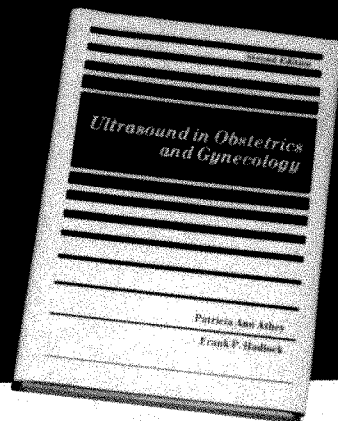
**HOW SUPPLIED:** Mycelex-G 500 mg Vaginal Tablets are white, bullet shaped, uncoated tablets, coded with Miles on one side and 097 on the other, supplied as a single 500 mg tablet with plastic applicator and patient instructions.

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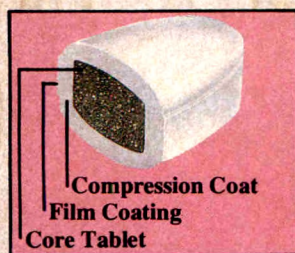
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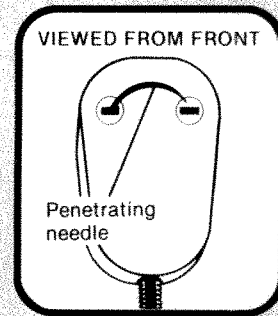
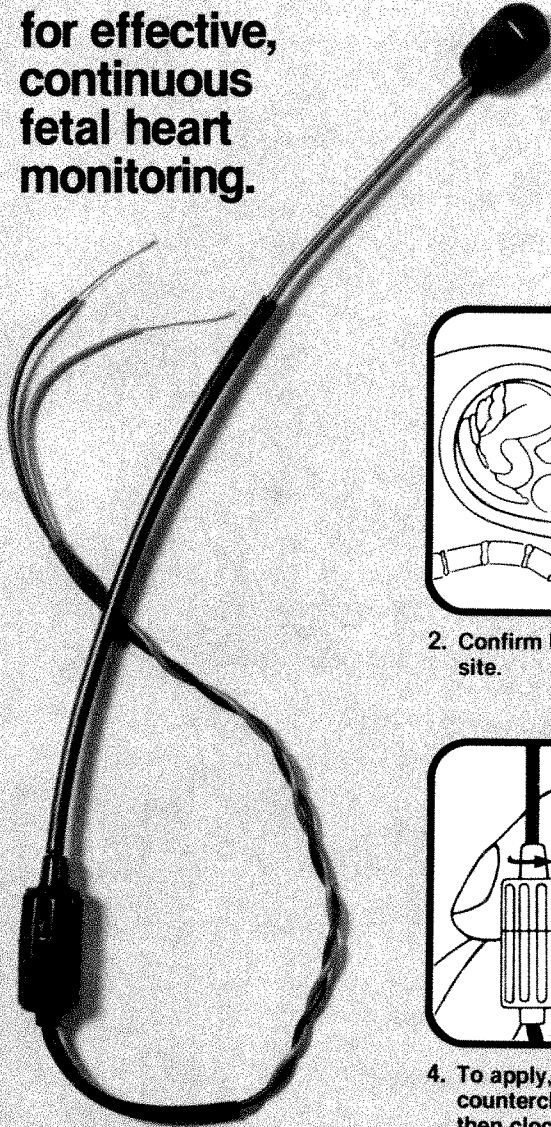
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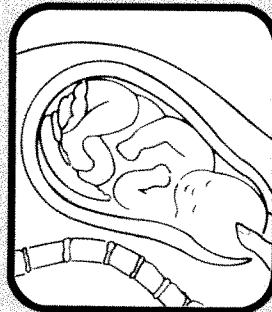


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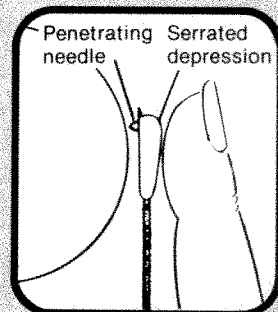
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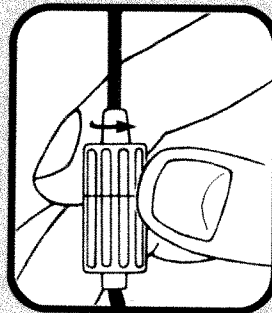
1. Once the cervix is sufficiently dilated insert the electrode housing.



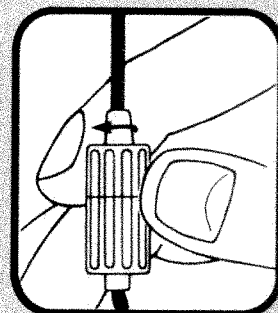
2. Confirm best scalp site.



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4. To apply, rotate counterclockwise and then clockwise.



5. To remove, rotate counterclockwise.

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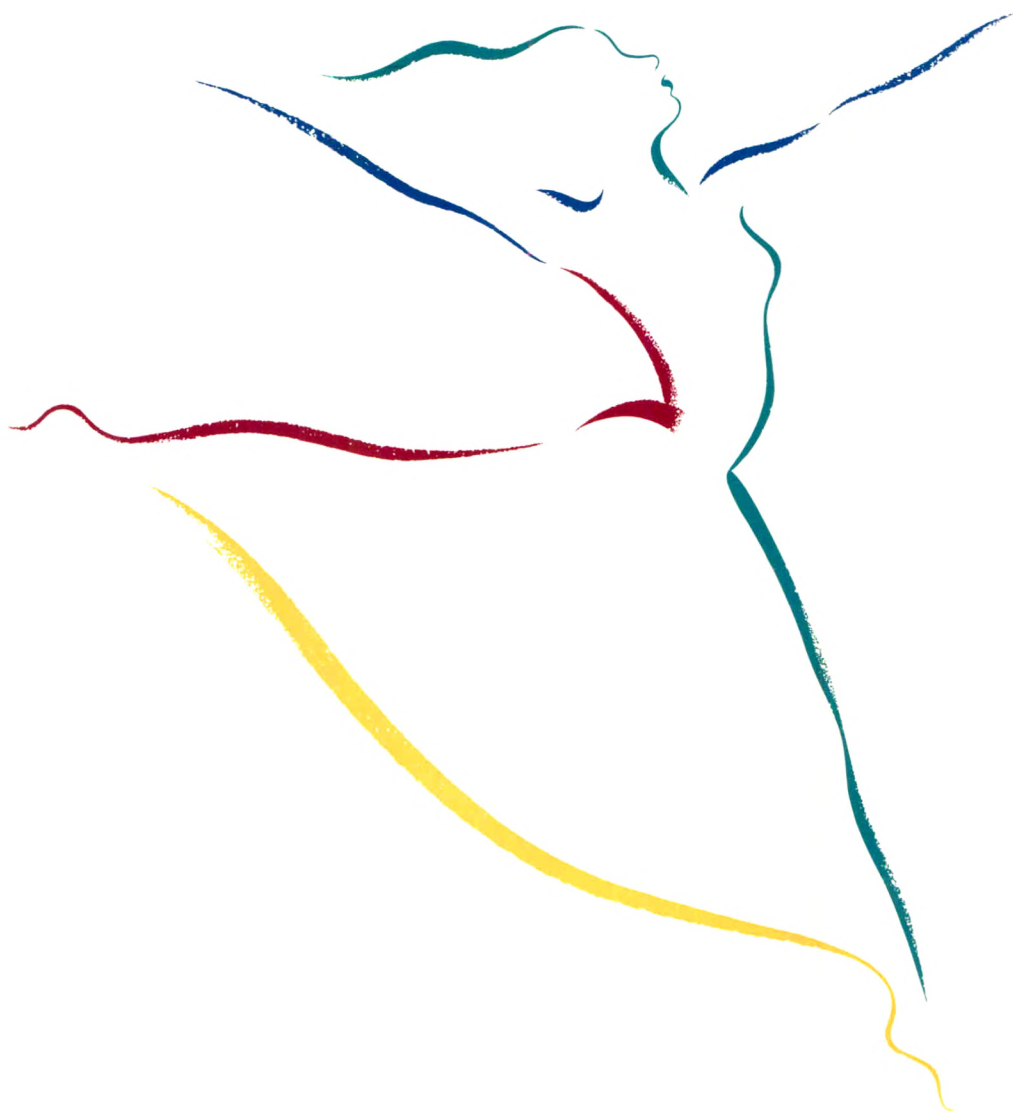
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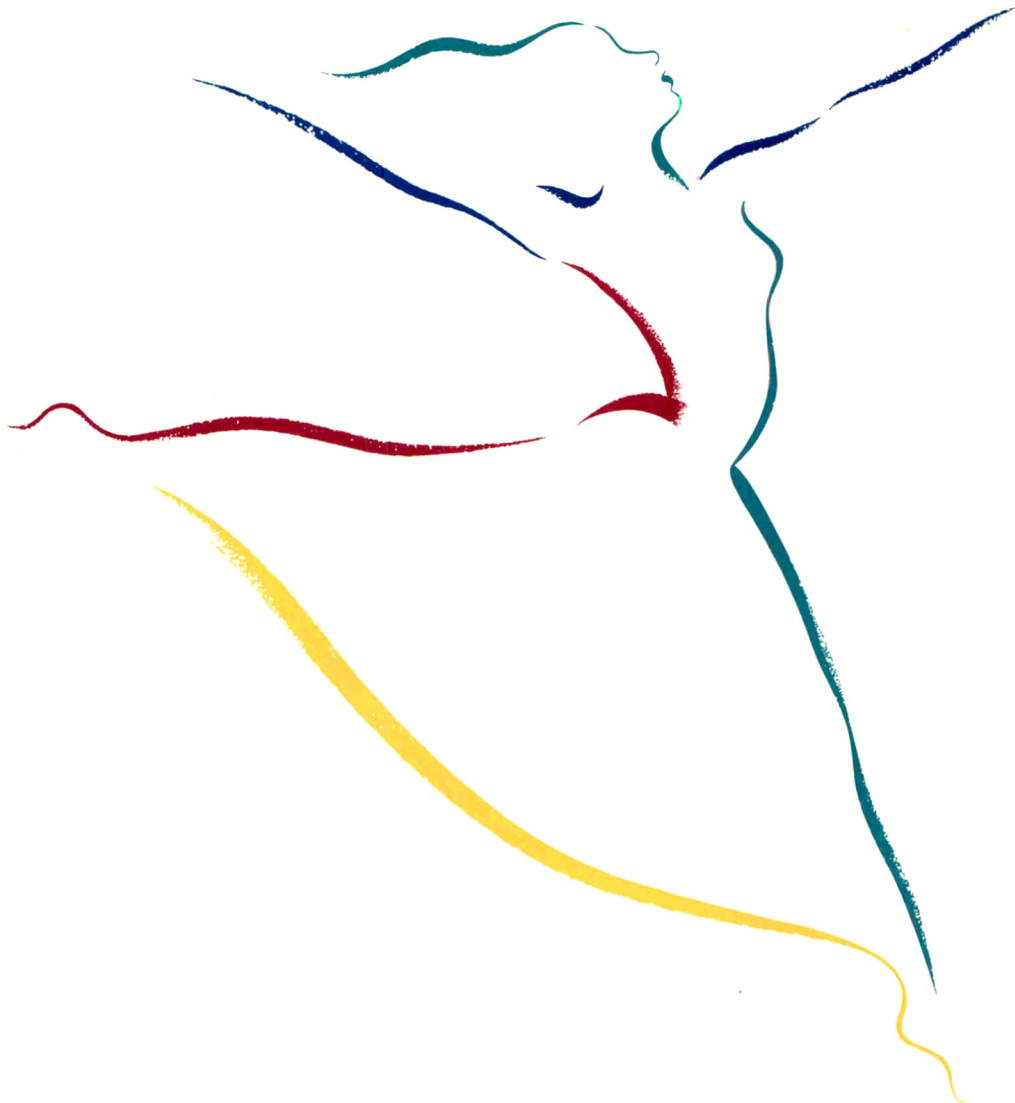
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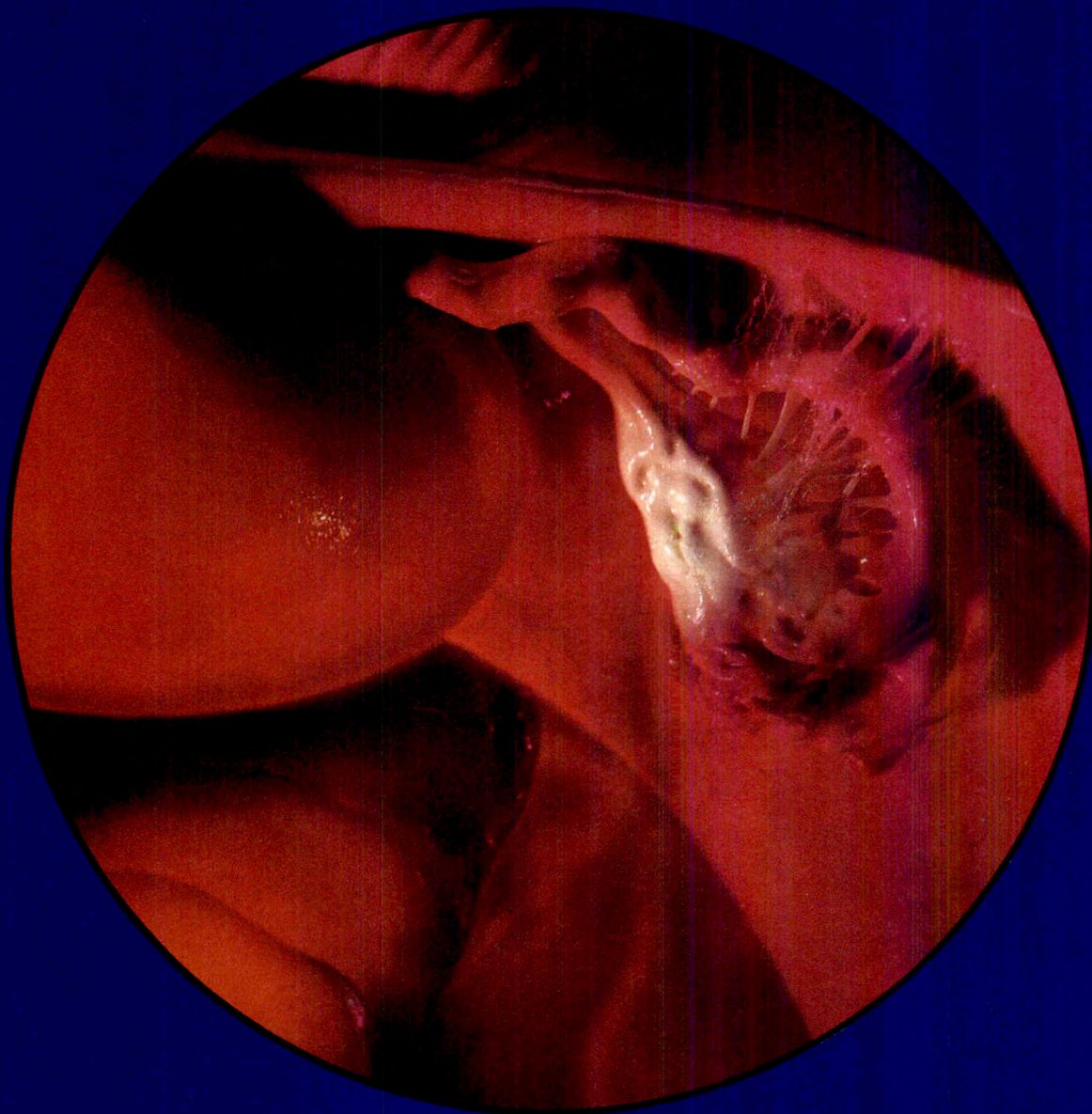
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**SEPTICEMIA** caused by *Strep. pneumoniae* (formerly *D. pneumoniae*), *Staph. aureus* (penicillinase and non-penicillinase producing), *E. coli*, *Klebsiella* species, and *Bacteroides* species including the *B. fragilis* group.

**BONE AND JOINT INFECTIONS** caused by *Staph. aureus* (penicillinase and non-penicillinase producing).

**SKIN AND SKIN STRUCTURE INFECTIONS** caused by *Staph. aureus* (penicillinase and non-penicillinase producing), *Staph. epidermidis*, streptococci (excluding enterococci, e.g., *Strep. faecalis*), *E. coli*, *P. mirabilis*, *Klebsiella* species, *Bacteroides* species including the *B. fragilis* group, *Clostridium* species, *Peptococcus* species, and *Peptostreptococcus* species.

Although appropriate culture and susceptibility studies should be performed, therapy may be started while awaiting these results. Cefoxitin is not active *in vitro* against most strains of *Pseudomonas aeruginosa* and enterococci (e.g., *Strep. faecalis*) and many strains of *Enterobacter cloacae*. Methicillin-resistant staphylococci are almost uniformly resistant to cefoxitin.

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**Warnings:** BEFORE THERAPY IS INSTITUTED, CAREFUL INQUIRY SHOULD BE MADE TO DETERMINE PREVIOUS HYPERSENSITIVITY REACTIONS TO CEFOTILIN, CEPHALOSPORINS, PENICILLINS, OR OTHER DRUGS. GIVE WITH CAUTION TO PENICILLIN-SENSITIVE PATIENTS. ANTIBIOTICS SHOULD BE ADMINISTERED WITH CAUTION TO ANY PATIENT WHO HAS DEMONSTRATED SOME FORM OF ALLERGY, PARTICULARLY TO DRUGS. IF AN ALLERGIC REACTION TO CEFOTILIN OCCURS, DISCONTINUE THE DRUG. SERIOUS HYPERSENSITIVITY REACTIONS MAY REQUIRE EPINEPHRINE AND OTHER EMERGENCY MEASURES.

**Pseudomembranous colitis, from mild to life-threatening in severity, has been reported with virtually all antibiotics (including cephalosporins); therefore, it is important to consider its diagnosis when diarrhea develops in association with antibiotic use.** Broad-spectrum antibiotics alter normal flora of colon and may permit overgrowth of clostridia; a toxin produced by *Clostridium difficile* is a primary cause of antibiotic-associated colitis. Mild cases may respond to drug discontinuance alone; in more severe cases, management may include sigmoidoscopy, appropriate bacteriological studies, fluid, electrolyte and protein supplementation, and use of a drug such as oral vancomycin; isolation of the patient may be advisable. Other causes of colitis should also be considered.

**Precautions: General**—Total daily dose should be reduced in patients with reduced urinary output due to renal insufficiency because high and prolonged serum antibiotic concentrations can occur from usual doses. Prescribe with caution in patients with a history of gastrointestinal disease, particularly colitis. Prolonged use may result in overgrowth of nonsusceptible organisms; repeated evaluation of the patient's condition is essential. If superinfection occurs, take appropriate measures.

**Drug Interactions**—Increased nephrotoxicity has been reported following concomitant administration of cephalosporins and aminoglycoside antibiotics.

**Drug/Laboratory Test Interactions**—High concentrations (>100 mcg/mL) may interfere with measurement of serum and urine creatinine levels by the Jaffe reaction and produce false increases of modest degree in creatinine levels reported; serum samples should not be analyzed for creatinine if withdrawn within 2 hours of cefoxitin administration. High concentrations may interfere with measurement of urinary 17-hydroxy-corticosteroids by the Porter-Silber reaction and produce false increases of modest degree in levels reported. A false-positive reaction for glucose in urine has been observed with CLINITEST<sup>®</sup> reagent tablets.

**Carcinogenesis, Mutagenesis, Fertility Impairment**—No long-term animal study has been performed on carcinogenic or mutagenic potential. Rat studies at approximately three times maximum recommended human dosage revealed no effects on fertility or mating ability.

**Pregnancy Category B**—Reproduction studies in rats and mice did not reveal teratogenic or fetal toxic effects, although fetal weights were slightly decreased. In rabbits, cefoxitin was associated with a high incidence of abortion and maternal death, neither considered teratogenic. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

**Nursing Mothers**—Excreted in human milk: Exercise caution.

**Pediatric Use**—Safety and efficacy in infants from birth to three months have not yet been established. In children three months and older, higher doses have been associated with increased incidence of eosinophilia and elevated SGOT.

**Adverse Reactions:** The most common adverse reactions have been local reactions following intravenous or intramuscular injection. Other adverse reactions have been encountered infrequently. **Local Reactions**—Thrombophlebitis with intravenous administration; pain, induration, and tenderness after intramuscular injections. **Allergic Reactions**—Rash (including exfoliative dermatitis), pruritus, eosinophilia, fever, and other allergic reactions. **Gastrointestinal**—Symptoms of pseudomembranous colitis during or after treatment and, rarely, nausea and vomiting. **Blood**—Transient eosinophilia, leukopenia, neutropenia, hemolytic anemia, and thrombocytopenia; a positive direct Coombs test may develop in some individuals, especially those with azotemia. **Liver Function**—Transient elevations in SGOT, SGPT, serum LDH, and serum alkaline phosphatase. **Renal Function**—Elevations in serum creatinine and/or blood urea nitrogen levels and, rarely, acute renal failure.

**Note:** In group A beta-hemolytic streptococcal infections, therapy should be maintained for at least 10 days to guard against the risk of rheumatic fever or glomerulonephritis. In staphylococcal and other infections involving a collection of pus, surgical drainage should be carried out where indicated. Intramuscular injections should be well within the body of a relatively large muscle such as the upper outer quadrant of the buttock (i.e., gluteus maximus); aspiration is necessary to avoid inadvertent injection into a blood vessel. The total daily dosage in infants and children should not exceed 12 grams.

**How Supplied:** Sterile cefoxitin sodium in vials and infusion bottles containing 1 gram or 2 grams cefoxitin equivalent and in 10-gram bulk bottles.

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The length of text material (introduction through Comment section) in regular manuscripts accepted for publication normally ranges from 750 to 4200 words (an average of 2000 words). A 4200 word text can seldom be accepted, especially if tables and figures are included. The average manuscript of 2000 words of text with abstract, 3 tables with captions, 2 figures with legends, and references makes a 5.7 page article in the JOURNAL. The 2000 words of text alone make approximately 8 pages of manuscript typed double spaced with the required 1 inch margins (approximately 250 words per page). A table or figure that occupies both columns of half a JOURNAL page is equivalent to approximately 500 typed words in manuscript. Thus if a greater number of illustrations and tables are used, the length of the text should be adjusted accordingly.

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**Case reports and brief clinical and basic science communications.** Limit of 700 words, 2 references. Include abstract of 50 words maximum, 3 to 5 key words/phrases for indexing purposes, and short title. If tables and/or figures are used, an equivalent number of words must be deducted from the total (see "Estimating Length of Manuscript").

**Current Investigation.** Same requirements as for a regular article.

**Clinical Opinion.** Limit of 3000 words, 16 references. Include abstract of 50 to 150 words, 3 to 5 key words/phrases, and short title. Submit to Dr. Zuspan.

**Current Development.** Limit of 6000 words. Include abstract of 50 to 150 words, 3 to 5 key words/phrases, and short title. Submit to Dr. Zuspan.

**Correspondence.** Two types of correspondence will be considered for publication. (1) A Letter to the Editors commenting on an article that has appeared in the JOURNAL should be brief and directly related to the published article. The editorial staff reserves the right to shorten letters if necessary and to make minor editorial alterations without reference to the writer. Letters may be published together with a reply from the original author. As space for letters is limited, only a selection of letters submitted may be published. (2) A brief case presentation or a short report of a pertinent observation in the form of a Letter to the Editors will be considered for publication. All letters should be typed double spaced. Letters should be sent to Dr. Zuspan.

**Announcements.** Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of \$60.00 U.S. and the fee

must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to The C. V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146.

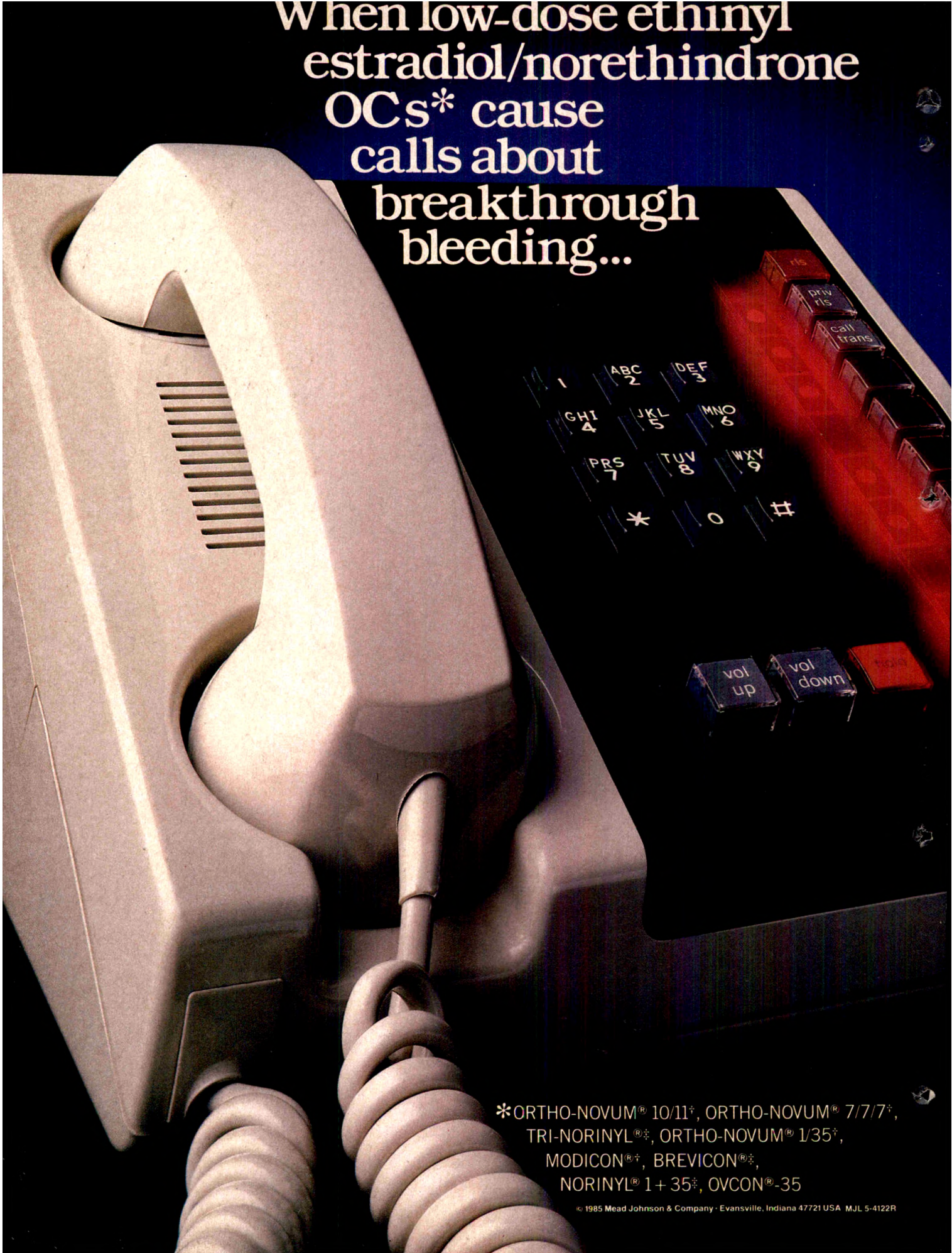
**Books.** Books received will be listed in the JOURNAL. They should be sent to Dr. Gerbie. No books will be returned.

**Reprints.** Reprints of articles must be obtained from the author. The corresponding author will receive a price schedule and order form at the time of publication. Reprints in quantities must be ordered from the Publisher with the author's consent.

### Checklist

- Letter of submission
- Copyright transfer letter
- Original and two xerographic copies of manuscript
- Title page
- Title of article
- Full name(s) and affiliations of author(s)
- Author to whom correspondence is to be sent
- Reprint request line or line stating reprints not available
- Short title
- Abstract (double spaced), 3 to 5 key words/phrases
- Article proper (double spaced)
- References (double spaced), on a separate sheet
- Legends (double spaced), on a separate sheet
- Tables (double spaced), each on a separate sheet
- Illustrations, properly labeled (three copies of glossy prints)
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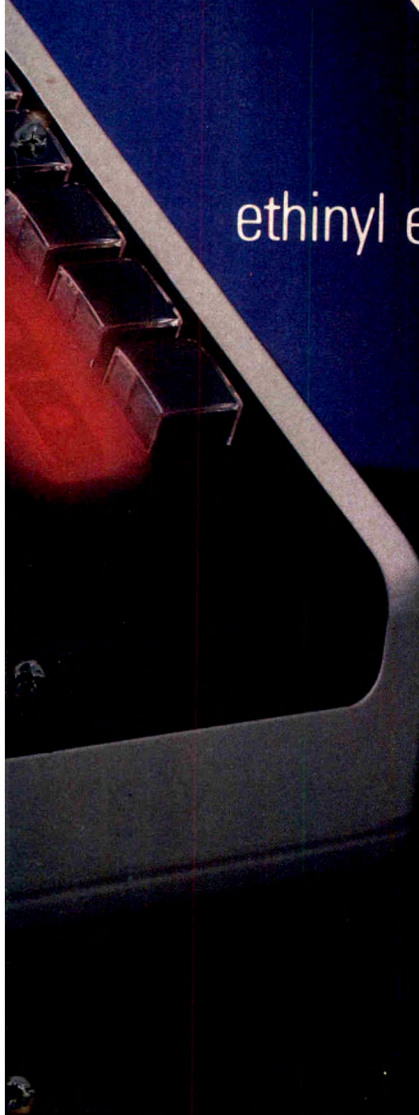


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1. Mishell DR Jr: Oral steroids, in Mishell DR Jr, Davajan V(eds): *Reproductive Endocrinology, Infertility and Contraception*. Philadelphia, FA Davis Co, 1979, p 489.

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**Contraindications:** Oral contraceptives should not be used in women with any of the following conditions: 1) Thrombophlebitis or thromboembolic disorders. 2) A past history of deep vein thrombophlebitis or thromboembolic disorders. 3) Cerebral vascular or coronary artery disease. 4) Known or suspected carcinoma of the breast. 5) Known or suspected estrogen dependent neoplasia. 6) Undiagnosed abnormal genital bleeding. 7) Known or suspected pregnancy. 8) Benign or malignant liver tumor which developed during the use of oral contraceptives or other estrogen-containing products.

#### Warnings:

**Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. Women who use oral contraceptives should be strongly advised not to smoke. Use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.**

1) **Thromboembolic Disorders and Other Vascular Problems.** An increased risk of thromboembolic and thrombotic disease associated with use of oral contraceptives is well established. Three principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of oral contraceptives are 4 to 11 times more likely than nonusers to develop these diseases without evident cause. In a study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater. An increased risk of myocardial infarction associated with the use of oral contraceptives has been reported. Studies conducted in the United Kingdom found that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of pre-eclampsia toxemia) the higher the risk of developing myocardial infarction, regardless of whether the patient was an oral contraceptive user or not. Oral contraceptives, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that oral contraceptive users who do not smoke (smoking considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction compared to nonusers who do not smoke. Oral contraceptive users who smoke have about a 5-fold increased risk of fatal infarction compared to users who do not smoke but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. The amount of smoking is a very important factor. British investigators concluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in oral contraceptives. Preparations containing 400 mcg, or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. The relative risk of thromboembolism associated with progestin-only oral contraceptives has not been determined. Cases of thromboembolic disease have been reported in women using progestin-only products, and they should be presumed to be risk-free. The overall excess mortality rate annually from circulatory diseases for oral contraceptive users was estimated to be 20 per 100,000 (ages 15-34, 5/100,000; ages 35-44, 33/100,000; ages 45-49, 140/100,000), the risk being concentrated in older women, in those with a long duration of use, and in cigarette smokers. The highest risk was found in heavy cigarette smokers (15 or more cigarettes per day) who used oral contraceptives and were aged 40 or older. Women who smoke should be advised not to use oral contraceptives. The use of oral contraceptives in women over age 40 with other risk factors is not recommended. The mortality associated with all of the methods of birth control is low compared to the risk of childbirth, with the exception of oral contraceptive users who smoke and are over age 40. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with oral contraceptives increases with age after approximately age 30 and for myocardial infarction is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of pre-eclampsia toxemia, and especially by cigarette smoking. The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. If feasible oral contraceptives should be discontinued at least 4 weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization. 2) **Ocular Lesions.** There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of oral contraceptives. Discontinue the medication if there is unexplained sudden or gradual, partial or complete loss of vision; sudden onset of proptosis or diplopia; papilledema; or retinal vascular lesions, and institute appropriate diagnostic and therapeutic measures. 3) **Carcinoma.** Long term administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina and liver in humans. One study investigated the first 21 cases of endometrial adenocarcinoma in women on oral contraceptives reported to a registry. Of those women without predisposing risk factors for this disease, nearly all occurred in women who had used a sequential oral contraceptive. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestin-only oral contraceptives. No increase in breast cancer in women taking oral contraceptives has been reported although one study reported an increased risk of breast cancer in subgroups of women treated using oral contraceptives with documented benign breast disease. There is at present no confirmed evidence from human studies of an increased risk of cancer associated with oral contraceptives. Close clinical surveillance of all women taking oral contraceptives is essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use oral contraceptives. 4) **Hepatic Adenoma.** Benign hepatic adenomas have been found to be associated with the use of oral contraceptives. One study reported a higher risk associated with oral contraceptive formulations with high hormonal potency. Although benign and rare, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short term as well as long term users of oral contraceptives. Two studies relate the risk with duration of contraceptive use, the risk being much greater after 4 or more years of oral contraceptive use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass, or shock. A few cases of hepatocellular carcinoma have been reported in women taking oral contraceptives. The relationship of these drugs to this type of malignancy is not known at this time. 5) **Use in Pregnancy, Birth Defects in Offspring and Malignancy in Female Offspring.** Fetal abnormalities have been reported to occur in the offspring of women who have taken progestogens and/or estrogens during pregnancy. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. A high percentage of such exposed women (30% to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Similar data are not available with the use of other estrogens but it cannot be presumed that they would not induce similar changes. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 in 1000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual

abortion. There is considerable evidence that estrogens are ineffective for these indications and there is no evidence from well-controlled studies that progestins are effective for these uses. Increases in chromosomal aberrations have been reported in women who become pregnant soon after ceasing oral contraceptive therapy. Embryos with these anomalies are virtually always spontaneously aborted. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping oral contraceptives is unknown. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period and further use of oral contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is confirmed the patient should be apprised of the potential risks to the fetus and the advisability of pregnancy continuation should be discussed in light of these risks. It is also recommended that women who discontinue oral contraceptives with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend three months. Administration of progestin-only or progestin-estrogen combinations to induce withdrawal bleeding should not be used as a test for pregnancy. 6) **Gall Bladder Disease.** Studies have reported an increased risk of surgically confirmed gallbladder disease appearing after one year of oral contraceptive use and doubling of the risk after 4 or 5 years of use. 7) **Carbohydrate and Lipid Metabolic Effects.** A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. For this reason, prediabetic and diabetic patients should be carefully observed while receiving oral contraceptives. Increased serum levels of triglycerides and total phospholipids have been observed in oral contraceptive users. The clinical significance of this observation is unknown at this time. 8) **Elevated Blood Pressure.** An increase in blood pressure has been reported in women receiving oral contraceptives. The prevalence of hypertension in oral contraceptive users may be no higher than nonusers in the first year of oral contraceptive use but increases with longer exposure and in the fifth year of use is two and one-half to three times the reported prevalence in the first year. Women who previously had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given oral contraceptives. 9) **Headaches.** The onset or exacerbation of migraine or development of a new pattern which is recurrent, persistent or severe, requires discontinuation of oral contraceptives and investigation of the cause. 10) **Bleeding Irregularities.** Breakthrough bleeding, spotting and amenorrhea are frequent reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or change to another formulation may solve the problem. Changing to an oral contraceptive with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of oral contraceptives. Women with these premenstrual problems should be advised of this possibility and encouraged to use other contraceptive measures. 11) **Ectopic Pregnancy.** Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. However, in oral contraceptive failures, the ratio of ectopic to intrauterine pregnancies is higher than in women not using oral contraceptives since the drugs are more effective in preventing intrauterine rather than ectopic pregnancy. The higher ectopic-intrauterine ratio has been reported with both combination products and progestin-only oral contraceptives. 12) **Breast Feeding.** A small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The long-range effect to the nursing infant cannot be determined at this time.

**Precautions:** 1) A complete medical and family history should be taken prior to the initiation of oral contraceptives. Examination should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, oral contraceptives should not be prescribed for longer than 1 year without another physical examination being performed. 2) Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3) Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. 4) Because oral contraceptives may cause some degree of fluid retention, conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, cardiac or renal insufficiency or asthma require careful observation. 5) Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving oral contraceptive therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6) Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7) Oral contraceptive users may have disturbances in normal tyrosine metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8) Serum folate levels may be depressed by oral contraceptive therapy. This may complicate subsequent pregnancy with regard to folate deficiency. 9) The pathologist should be advised of oral contraceptive therapy when relevant specimens are submitted. 10) Certain endocrine and liver function tests and blood components may be affected by estrogen-containing oral contraceptives: a. increased sulfobromophthalen retention; b. increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability; c. increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T<sub>4</sub> by column, or T<sub>4</sub> by radioimmunoassay. Free T<sub>3</sub> resin uptake is decreased, reflecting the elevated TBG. Free T<sub>4</sub> concentration is unaltered; d. decreased pregnandiol excretion; e. reduced response to metoprolol test. 11) The active yellow tablets and the inert green tablets in Ovcon-50 (21 and 28 day regimens) and the inert green tablets in the 28 day regimen of Ovcon-35 contain FD&C Yellow No. 5 (tartrazine) which may cause allergic-type reactions (including bronchial asthma) in certain susceptible individuals. Although the overall incidence of FD&C Yellow No. 5 (tartrazine) sensitivity in the general population is low, it is frequently seen in patients who also have aspirin hypersensitivity.

**Information for the Patient:** Detailed Patient Labeling has been prepared for use by the patient and has been made available for distribution by the pharmacist.

**Drug Interactions:** Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

**Adverse Reactions:** An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see **Warnings**): Thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gall bladder disease, congenital anomalies. There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis; benign hepatomas; neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug related: Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10% or less of patients during the first cycle (other reactions, as a general rule, are seen much less frequently or only occasionally); gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding; spotting; change in menstrual flow; dysmenorrhea; amenorrhea during and after treatment; temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes (tenderness, enlargement, and secretion); change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; changes in corneal curvature (steepening); intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives, and the association has been neither confirmed nor refuted: Premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria.

**Acute Overdosage:** Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

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## EDITORIAL

### Change and consistency

A change in the JOURNAL is announced. Starting with this issue, January, 1986, the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY will be published once a month, two volumes a year, rather than semimonthly, three volumes a year, as it has been since 1962.

The change was made following the recommendation of our Advisory Committee on Policy. The Committee was of the opinion that, with all the many journals available and the restricted time for selective and general reading, monthly publication of the JOURNAL would be more desirable for and more acceptable to our readers.

Again with the readers and authors in mind, the JOURNAL will publish approximately the same number of articles and text pages in the 12 issues each year as were published in 24 issues a year in the past.

As this is a major change, a study was initiated to determine the previous changes made in the JOURNAL. This promptly demonstrated the need to extend the study to include the history of the *American Journal of Obstetrics and Diseases of Women and Children*. In 1920, The C. V. Mosby Company acquired the *American Journal of Obstetrics and Diseases of Women and Children* from its Publisher, William Wood & Co., New York, New York, and made only one change. They changed its name to the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY and retained its Editor, George W. Kosmak, M.D., who had served since 1912 and who continued as Editor of the newly named JOURNAL until 1952. The Editor and The C. V. Mosby Company fully intended to carry forward the same objective and aims of the earlier publication.

The *American Journal of Obstetrics and Diseases of Women and Children* was founded in 1868. It was owned and edited by E. Naeggerath, M.D., Late Professor of Obstetrics and Diseases of Women and Children, New York Medical College, and B. F. Dawson, M.D., Lecturer on Uterine Pathology in the Medical Department of the University of New York.

The objective of the original Editors was to supply the want long felt by many practitioners for a Periodical that would be devoted exclusively to obstetrics and the diseases of women and children. The aims were to bring to the readers the latest and accepted views and teachings of recognized authorities on their subjects, and the publication of the Transactions of the major obstetrical

and gynecological societies, and the reports from eminent foreign authors, which would afford valuable and practical information on a broad range of subjects. In addition, it would furnish extracts, reviews, and a general synopsis of the same.

The emphasis placed upon publication of the Transactions of obstetrical and gynecological societies is indicated by the publication of the Transactions of the New York Obstetrical Society in the first issue of the *Journal*, May, 1868. In 1872, publication of the Transactions of the Philadelphia Obstetrical Society was initiated.

An Editorial in the August, 1876, issue of the *American Journal of Obstetrics and Diseases of Women and Children* briefly described the formation of the American Gynecological Society and announced that the full reports of each annual meeting would be published by the Society on its own in an annual bound volume of Transactions, but the *Journal* would publish a synopsis of the proceedings each year in the *Journal* under the title of Transactions of the American Gynecological Society. The first Transactions appear in this same issue of August, 1876.

In 1888, the *Journal* started publication of the Transactions of the American Association of Obstetricians and Gynecologists. The Association also produced, on its own, an annual separate bound Transactions volume and had it printed for the Association. Recently, the American Gynecological Society and the American Association of Obstetricians and Gynecologists merged to form the American Gynecological and Obstetrical Society. The first Transactions of the combined society were published in 1983.

Between 1876 and 1888, the *Journal* began publishing the Transactions of the following societies: Cincinnati Obstetrical Society, 1877; Boston Obstetrical Society, 1879; German Gynecological Society, 1879; Obstetrical Society of London, 1881; Obstetrical Society of Edinburgh, 1882; Obstetrical Society of Dublin, 1882; Obstetrical and Gynecological Society of Washington, 1883; and Chicago Gynecological Society, 1885.

The *Journal's* interest in publishing meritorious foreign articles is evidenced by the statement in an Editorial in the first issue of May, 1868. One of the Editors was in Europe for several months and, while there, would make arrangements to secure valuable foreign



material for future issues of the *Journal*; and this he did.

The objective and aims of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY, since it was first published under this name by The C. V. Mosby Company, October, 1920, have been a continuation of those established by the *American Journal of Obstetrics and Diseases of Women and Children*. The objective is to be of service to the readers, to aid them in keeping pace with new developments and advancements in the increasingly broad areas of obstetrics and gynecology and related fields, and to provide both clinicians and scientists a medium for wide dissemination of their studies and experiences. We believe such a united effort will contribute to and advance our Specialty. The aims are to continue to publish selected, independently submitted manuscripts that contain valuable material and are prepared by domestic and foreign authors, and to emphasize the publications of Transactions of various societies.

Clinical investigation and basic science research in and related to our specialty have expanded greatly. This fact and our desire to continue to publish articles of practical clinical importance have led us to publish an average of 2890 text pages per year for each of the last six years. The change from a monthly to a semi-monthly publication of the JOURNAL, starting in 1962, was made to provide more pages for this purpose.

The balance attained between three types of articles published in the JOURNAL in the last six years averaged as follows: clinical articles, 48%; clinical investigation articles, 32%; basic science articles, 20%. The latter two have value because the findings and results described oftentimes become an essential and practical element in clinical practice.

The emphasis placed on publication of Transactions since 1868 has been maintained. We continue to publish the Transactions of some of the societies that were published by the *American Journal of Obstetrics and Diseases of Women and Children*. We believe they are important and have added new ones over the years. The relationship between the societies and the JOURNAL has been excellent and we appreciate this very much.

The efforts to attract authors from foreign countries, which began in 1868, were increased in the late 1950s when Dr. Howard C. Taylor, Jr., was Editor in Chief. In 1961, we received 69 foreign manuscripts; in 1984, we received 408 from 44 foreign countries.

A formal peer review process for all manuscripts was developed many years ago. The consultants are qual-

ified authorities in their fields and have been extremely helpful in our efforts to produce a JOURNAL of quality.

To give the profession a voice in matters pertaining to the JOURNAL an Advisory Committee on Policy was established years ago and meets annually. The Editors and the Publisher, The C. V. Mosby Company, thank the present and all past members of the Committee for their excellent participation, advice, and support.

A remarkable consistency has been maintained in the JOURNAL for a span of 117 years. Since 1868, the readers have been consistently provided articles with a broad range of topics that have permitted them to keep pace with new developments as they occur in obstetrics, gynecology, fetus, placenta, and newborn, and related areas. The publication of Transactions of societies has continued unabated from the first publication in May, 1868, to the present and actually the number has increased. The effort to provide readers with important and valuable reports from foreign sources, which began with the founding of the *American Journal of Obstetrics and Diseases of Women and Children* in 1868, has been continuous to the present.

Since 1873, there have been only two Publishers, William Wood & Co., New York, New York, for 47 years as Publisher of the *American Journal of Obstetrics and Diseases of Women and Children*, and The C. V. Mosby Company, St. Louis, Missouri, as Publisher of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY for 65 years. This consistent service indicates the esteem with which both Publishers held and still hold the JOURNAL.

The transition of the JOURNAL from one Publisher to the other, in 1920, was made easy and consistency was maintained by the retention of the Editor, Dr. George W. Kosmak, who served as Editor of the *American Journal of Obstetrics and Diseases of Women and Children* for 8 years and as Editor of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY for the next 33 years, a total of 41 years.

The information gathered from our study clearly shows the JOURNAL's changes that have occurred are consistent with the trends in medicine, the progress and advancements in obstetrics and gynecology and related fields of specialization in particular, the changes in the practice and science of our specialty, and the changing of times. Our most recent change, to a monthly publication schedule, is made with the Editors' desire to be of greater service and benefit to our readers.

*The Editors and Publisher*



### Clinical Opinion

#### Preterm labor: Its diagnosis and management

Bernard Gonik, M.D., and Robert K. Creasy, M.D.

Houston, Texas

Preterm labor and delivery remain a significant problem in contemporary obstetric practice. Although the exact cause remains unclear, it is most likely to be multifactorial in nature. No satisfactory screening tool or marker currently exists to firmly establish the diagnosis of impending labor. However, epidemiologic and historical variables associated with preterm delivery show some promise in this regard and are currently being evaluated in preterm prevention programs. Appropriate management of preterm labor mandates early recognition of subtle signs and symptoms; successful therapy is dependent on this issue. The approach to the clinical management of the patient in preterm labor used at our institution is described. Therapy with  $\beta$ -adrenergic receptor agonists is currently the recommended pharmacologic treatment of this disorder. A review of other tocolytic agents and their usefulness in the management of preterm labor are presented. (AM J OBSTET GYNECOL 1986;154:3-8.)

**Key words:** Preterm labor, obstetrics,  $\beta$ -adrenergic receptor agonists

National, regional, and local perinatal data provide convincing evidence of improved maternal and neonatal health during the past several decades. Conversely, preterm delivery rates in the United States, over the same period of time, have remained relatively unaltered, in the range of 7% to 8%.<sup>1</sup> The impact of this finding can best be appreciated by the recognition that four of the six leading causes of infant death from 1970 to 1978 are almost exclusively associated with the premature neonate.<sup>1</sup>

The World Health Organization uses, as its definition of a preterm infant, any neonate born prior to 37 completed weeks of gestation. No clear standards are established for the lower limits of this definition although, by tradition, an abortus is considered in any gestation less than 20 weeks. For the purpose of this discussion, we define the preterm infant as one who is born after 20 completed weeks from the first day of the last menstrual period and before 37 completed weeks of gestation. Although a birth weight of <2500 gm has been used in the past synonymously with this definition, a portion of these neonates actually represent growth retardation. Since perinatal morbidity and mortality issues are different for these two types of neonates, this distinction is important in the evaluation of recent lit-

erature pertaining to preterm labor and delivery management.

The mechanisms involving the initiation of labor both before and at term are still poorly understood. An extensive review of this topic has recently been presented by Huszar and Naftolin,<sup>2</sup> who stressed three relatively distinct areas to be considered when addressing this subject. These include: (1) cervical maturational changes, (2) status of the fetal membranes, and (3) myometrial activity. Although the interrelationship of these factors is obvious, regulation of the individual components may not be identical. In addition, one can clinically identify pathologic alterations in each of these factors in isolation. From a practical perspective, the management of preterm labor requires a combined knowledge of all of these factors. However, the above issues support the multitietologic approach to the onset of preterm labor.

#### Prediction of preterm labor

Primary to the reduction in the preterm birth rate is the establishment of criteria for the prediction of preterm labor. Since the causative factors of this event have yet to be clearly defined, systems thus far used to predict preterm labor primarily depend on historical or epidemiologic variables and their association with preterm delivery.<sup>3</sup> None of these risk-scoring systems have reached the level of discrimination necessary to be used as primary tools in establishing protocols to prevent the initiation of preterm labor. In addition, because these systems rely heavily on past obstetric outcomes, they are better equipped to screen the multiparous patient.

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**Table I.** System for determining risk of spontaneous preterm delivery

<i>Points assigned</i>	<i>Socioeconomic factors</i>	<i>Previous medical history</i>	<i>Daily habits</i>	<i>Aspects of current pregnancy</i>
1	Two children at home Low socioeconomic status	Abortion × 1 Less than 1 yr since last birth	Works outside home	Unusual fatigue
2	Maternal age <20 yr or >40 yr Single parent	Abortion × 2	Smokes more than 10 cigarettes per day	Gain of <5 kg by 32 wk
3	Very low socioeconomic status Height <150 cm Weight <45 kg	Abortion × 3	Heavy or stressful work that is long and tiring	Breech at 32 wk Weight loss of 2 kg Head engaged at 32 wk Febrile illness
4	Maternal age <18 yr	Pyelonephritis		Bleeding after 12 wk Effacement Dilation Uterine irritability Placenta previa Hydramnios
5		Uterine anomaly Second-trimester abortion Diethylstilbestrol exposure Cone biopsy Preterm delivery Repeated second- trimester abortion		
10				Twins Abdominal surgical procedure

Adapted from Creasy RK, Gummer BA, Liggins GC. A system for predicting spontaneous preterm birth. *Obstet Gynecol* 55:692, 1980. Score is computed by adding the number of points given any item. The score is computed at the first visit and again at 22 to 26 weeks' gestation. A total score of  $\geq 10$  places patient at high risk of spontaneous preterm delivery.

A multi-institutional collaborative study is currently underway to evaluate the utility of a preterm prevention program that incorporates the risk assessment system presented in Table I. These types of programs help to focus attention and provide education to a variety of health care personnel which should ultimately improve any therapeutic intervention program.

With the advent of more sophisticated monitoring technology, normal and abnormal patterns of uterine activity are being established throughout the latter half of gestation. Earlier investigations suggested a progressive increase in uterine activity as gestation advances, and, in the patient destined to deliver preterm, a premature increase in this activity may occur. Katz et al.,<sup>4</sup> using a portable tocodynamometer, recently reported that there is significantly more uterine activity in the ambulatory patient who later develops preterm labor. The usefulness of this finding relates to its occurrence up to 8 weeks prior to actual development of clinical manifestations of labor. Although this biophysical screening tool shows promise, further studies are needed to help establish its specificity and sensitivity in predicting preterm labor.

Perhaps the most useful of all predictive indicators would be a biochemical marker that could be assayed serially in those patients thought to be at increased risk of preterm labor. Earlier work suggested that rising plasma levels of 17 $\beta$ -estradiol or falling or low levels of serum progesterone may be useful in this regard. However, Block et al.<sup>5</sup> were prospectively unable to dem-

onstrate any relationship between weekly serum estradiol or progesterone concentrations and the prediction of preterm labor. For the present time, no biochemical marker that can reliably improve one's ability to predict this event has been established.

#### Early detection of preterm labor

Once a patient is categorized as at risk of developing preterm labor, by whatever predictive marker, weekly visits to the clinician for a review of early symptoms and for cervical examination seem warranted. Herron et al.<sup>6</sup> demonstrated that such a prevention program yielded a decreased preterm delivery rate by identifying an increased number of candidates eligible for long-term tocolytic therapy. The value of serial pelvic examinations is further supported by Holbrook et al.,<sup>7</sup> who demonstrated the ability to identify 18% of high-risk subjects in premature labor by cervical examination alone, even without other symptoms. Additionally, they reported no increase in the rate of premature labor or infectious complications in this same study population as compared with a control preterm labor group not undergoing serial examinations. Thus no increased iatrogenic risks from preterm serial examinations of the cervix have thus far been identified.

#### Diagnosis of preterm labor

The ability to prevent preterm deliveries is largely a function of early recognition and intervention.<sup>8</sup> Although labor in its later stages is obvious, early symp-



**Table II.** Criteria for diagnosis of preterm labor<sup>3</sup>

Gestation 20 to 37 wk and Documented uterine contractions (4/20 min, 8/60 min)	
Ruptured membranes	or Intact membranes and Documented cervical change or Cervical effacement of 80% or Cervical dilatation of 2 cm

toms can be quite subtle and are frequently overlooked. In some series, only 10% to 20% of patients presenting in preterm labor are candidates for long-term tocolysis. In addition, other studies have demonstrated a 20% to 45% efficacy of placebo treatment alone in "preterm labor" patients, suggesting an inaccurate initial diagnosis. The predicament that confronts the clinician, therefore, is accurate early diagnosis without overzealous treatment of patients not truly in labor. The criteria used in this institution for the diagnosis of preterm labor are presented in Table II.

Castle and Turnbull<sup>8</sup> and later Boylan et al.<sup>9</sup> have used real-time ultrasonography to look at the presence or absence of fetal breathing movements and their relationship to labor. In the presence of fetal breathing, both term and preterm patients who initially presented with contractions were unlikely to progress into labor. The authors suggested that the early diagnosis of labor may be improved with the use of this ultrasonographic finding. Although this diagnostic tool may be of benefit in the future, further prospective studies are needed.

#### Treatment of preterm labor

Once the diagnosis of preterm labor is clearly established, definitive tocolytic therapy should be initiated on an emergent basis. Although some clinicians have used bed rest, hydration, and narcotic sedation as a screening tool to establish the diagnosis of labor, these methods should not be confused with therapeutic modalities. The cornerstone in the pharmacologic management of the preterm patient in labor is currently therapy with a  $\beta$ -adrenergic receptor agonist. The mechanism of action involves attachment of the drug to the  $\beta_2$ -adrenergic receptor with subsequent activation of adenylyl cyclase. The resultant increase in cyclic adenosine monophosphate decreases myosin light chain kinase activity, thereby interfering with the actin/myosin interaction. A variety of associated side effects have been reported with these agents, which are related to both  $\beta_1$ - and  $\beta_2$ -adrenergic responses throughout the body. We are currently using ritodrine hydrochloride as our  $\beta$ -adrenergic receptor agonist of choice. However, we are unaware of any study that clearly supports

**Table III.** Initial measures recommended when tocolytic therapy is initiated

Bed rest
Admission weight
Intravenous access line
Electrocardiogram
Baseline vital signs
Fetal monitor for contractions and fetal heart tones
Laboratory studies
1. Complete blood count
2. Electrolytes
3. Serum glucose
4. Colloid osmotic pressure
5. Urinalysis

the superiority of this agent versus any other in this class of drugs for efficacy or side effects.

When beginning tocolytic treatment we initially obtain baseline data as shown in Table III. Maternal weight is obtained daily, and hematocrit, serum potassium, glucose, and colloid osmotic pressure determinations are repeated every 6 to 12 hours during intravenous therapy. Intravenous administration of the  $\beta$ -adrenergic receptor agonist is currently the route of choice for initial tocolysis. An intravenous infusion pump should be used with the initial dose of 50 to 100 mcg/min. We use a 5% dextrose in water solution as our maintenance fluid because of recent evidence demonstrating an increased risk of pulmonary edema with isotonic saline solution.<sup>10</sup> In addition, we concentrate our tocolytic solution (300 mcg/500 ml) to reduce the amount of infused fluids. The starting dose can be gradually increased by 50 mcg/min every 10 to 15 minutes and titrated to the desired clinical response of uterine quiescence. The usual effective dose is between 150 and 350 mcg/min. If tocolysis is successful (less than one contraction every 10 to 15 minutes), we continue the infusion at that rate for 12 to 24 hours before switching to oral therapy.

A limited amount of pharmacokinetic data is currently available for this drug. In patients receiving continuous infusions, ritodrine appears to be eliminated from the maternal plasma at a biphasic rate; a rapid phase of elimination of the drug (half-life 40 to 60 minutes) is followed by a more prolonged disappearance phase (half-life 16 to 28 hours).<sup>11</sup> Oral ritodrine should be started prior to the discontinuation of intravenous therapy to maintain therapeutic levels. The exact timing of the first oral dose has yet to be determined; empirically we allow 30 minutes before discontinuing intravenous ritodrine.

Once oral therapy is begun, dosage determinations are made according to uterine activity, pelvic examination, and the maternal pulse rate. This latter clinical parameter has been shown to correlate with serum ritodrine concentrations and uterine inhibitory effects.<sup>12</sup>



We start with an oral dose of 20 mg every 2 hours. If the maternal pulse is  $<90$  bpm and contractions continue, on rare occasions we administer 30 mg every 2 hours to maintain tocolysis. Usually, we are able to decrease our frequency of administration to every 3 to 4 hours without a concomitant rise in uterine activity. We then attempt to sustain a resting pulse between 90 and 100 bpm. The usual dose will be 20 mg every 2 to 4 hours.

Ambulation of the patient may be attempted after 24 hours of oral therapy. This activity should be minimal and should equate with those needs the patient anticipates at home after discharge. No further blood work is needed once oral therapy has been started. The patient can be discharged home if uterine activity and cervical findings remain unchanged during the next 24-hour period. Here, again, the potential benefit of portable home monitoring of uterine activity shows promise.

The manufacturer's recommendations for surveillance of the patient on a regimen of intravenous ritodrine therapy include a baseline electrocardiogram and serial monitoring of glucose and electrolyte levels. Although both transient hypokalemia and transient hyperglycemia occur with the intravenous use of this medication, no specific therapy is warranted unless other medical complications exist, such as diabetes mellitus. We carefully restrict total administered fluids to 2500 ml/24 hr or less during intravenous treatment and for the first 24 hours of oral therapy, because of alterations in the renin-aldosterone control mechanisms that are known to occur with ritodrine and the recognized potential side effect of pulmonary edema. Although other investigators have suggested an upper limit of 1500 ml, we have not been as restrictive and have anecdotally seen only one documented case of pulmonary edema with our management. Of note, even at a 2500 ml restriction level, patients may still request excess oral fluids, which may relate to increased water imbibition seen in animal studies.

Concerns about the more serious maternal complications identified with  $\beta$ -adrenergic receptor agonists have led to the use of magnesium sulfate as a first- or second-line agent for tocolysis. The efficacy of this agent, however, has never been firmly established in an extensive controlled trial. However, observational data do suggest that uterine contractions can be eliminated when maternal serum levels of magnesium sulfate are in the range of 6 to 8 mEq/L (5.0 to 6.6 mg/100 ml) and the patient is in the early stages of labor.<sup>13</sup> These levels can generally be obtained by the initial administration of 4 to 6 gm of magnesium sulfate intravenously during 20 minutes followed by a maintenance dose of 1.5 to 3 gm/hr. Because of substantial patient-to-patient variations in serum levels, we determine

magnesium levels serially every 6 hours. The exact mechanism of action at the cellular level by which magnesium reduces uterine activity is speculative; in all probability magnesium competes with calcium ion, thereby inhibiting the actin/myosin interaction.

As magnesium sulfate has gained acceptance, an increasing number of undesirable side effects have also been identified, including serious problems such as pulmonary edema. In addition, in a preliminary report by Ferguson et al.<sup>14</sup> of an attempt to use magnesium sulfate in combination with ritodrine therapy to reduce untoward cardiovascular side effects, a surprisingly unacceptable increase in serious side effects was reported. This issue remains controversial and requires further evaluation. In the patient who becomes unresponsive to  $\beta$ -adrenergic receptor agonist therapy (perhaps because of uterine tachyphylaxis) or when a drug-related complication is identified, we use magnesium sulfate in combination with ritodrine or alone. Temporarily discontinuing ritodrine theoretically may help to increase the efficacy of  $\beta$ -adrenergic receptor agonists so that we can later reinstitute either intravenous or oral ritodrine and achieve maximal effects. In patients who are insulin-dependent diabetics, magnesium sulfate is preferentially used on our service to avoid the risk of uncontrolled hyperglycemia that can occur with  $\beta$ -adrenergic receptor agonist therapy.

Since the terminal process thought to be responsible for uterine contractions is production and release of prostaglandins, it is not surprising that prostaglandin synthetase inhibitors have been studied for tocolytic potential.<sup>15</sup> Their limited use, however, has stemmed from concerns regarding the potential for in utero narrowing or closure of the ductus arteriosus and perhaps persistent fetal circulation in the neonate. These effects have been anecdotally reported and appear to be most often associated with chronic administration beyond 34 weeks' gestation or in association with other risk factors such as sepsis or perinatal asphyxia.

The ease of administration of indomethacin via the oral or rectal route makes this agent an attractive alternative to  $\beta$ -adrenergic receptor agonist therapy. In our institution short-term administration of this agent for up to 72 hours has been occasionally used with the previable fetus when the gravid subject has developed resistance to traditional tocolytic therapy. Under these circumstances, the intermittent use of this agent in a dose of 25 mg every 4 to 6 hours may have merit. Further careful prospective work with this agent is warranted.

Other new clinical approaches to the treatment of preterm labor include the use of calcium channel-blocking agents. Since calcium appears to have a central role in the initiation of labor, agents such as nifedipine would seem reasonable candidates for tocolytic therapy.



Preliminary work indicates this drug may be useful as a tocolytic agent.<sup>16</sup> However, substantial concern is currently surfacing regarding the potential adverse effect this group of agents may have on the fetus. Although reports in the human have not demonstrated any overt toxicity, some animal work has demonstrated profound metabolic alterations in the fetus.<sup>17</sup> The use of these agents should be restricted to controlled clinical investigations. In our limited experience, oral administration of nifedipine in the previable and borderline viable fetus has been used with some suggested efficacy.

### Controversial management issues

The frequent concomitant use of corticosteroid therapy with  $\beta$ -adrenergic receptor tocolytics has raised some concerns regarding an increased risk of pulmonary edema. From a physiologic standpoint, both groups of agents have been associated with an increase in fluid retention, although betamethasone and dexamethasone are essentially devoid of mineralocorticoid activity. In addition, alterations in fluid fluxes have been implicated as the most influential factor in the development of this complication. However, Robertson et al.,<sup>18</sup> in a series of 343 women treated with intravenous  $\beta$ -adrenergic receptor agonist therapy, were unable to demonstrate any association between glucocorticoid administration and the development of pulmonary edema. It would seem prudent to institute efforts to reduce the risk of pulmonary edema in patients receiving both agents by maintaining close surveillance of intravenous and oral fluid therapy. Strict recording of intake and output and daily weighing of the patient are routine measures. This is particularly important during the first 24 to 72 hours of therapy since most cases of pulmonary edema have been reported to occur around 48 hours after initiation of tocolysis. Patients with twin gestations (who have an accentuated expansion of plasma volume) and those with low oncotic pressures ( $<15$  mm Hg) are at particular risk for this complication to develop.

In most institutions, rupture of the membranes is a relative contraindication to long-term tocolytic usage. This appears to represent concerns over masking incipient infection as well as the perceived lack of efficacy of these agents after membrane rupture. Although controlled studies are not available, our current policy is to limit the use of tocolytic agents under these circumstances to no more than 24 to 36 hours. During this time period we occasionally administer glucocorticoids in an effort to induce fetal lung maturation, recognizing that adequate data are lacking in this regard. On an individual basis, we have used continuous intravenous tocolytic therapy in patients perceived to have a "high" leak that has subsequently resealed or in those with a very preterm fetus. Under these circumstances,

an amniocentesis to rule out the possibility of a subclinical infection is usually attempted. Of particular concern is a recent collaborative study that suggested that the combination of premature rupture of the membranes and tocolytic therapy resulted in a higher incidence of respiratory distress syndrome than that found with these factors evaluated individually.<sup>19</sup> Before any clinical management protocol is established, this negative effect requires further evaluation and substantiation.

The issue of "prophylactic" tocolysis in patients thought to be at high risk for preterm delivery has received surprisingly little attention. Although it has been demonstrated that oral  $\beta$ -adrenergic receptor agonist therapy can decrease the recurrence rate of preterm labor, controlled prospective studies are lacking regarding the efficacy of these agents in preventing the initial occurrence in the patient at risk. Preliminary prophylactic studies with low-dose  $\beta$ -adrenergic receptor agonists have failed to demonstrate any significant delays in the onset of preterm labor in twin gestations. However, it is unclear from these study designs whether an adequate dose of the agent was administered. This preventative pharmacologic approach to the management of the high-risk patient requires further study with appropriate doses.

Once a patient has been started on a regimen of tocolytic therapy and labor has been successfully inhibited, the question arises as to the length of time the patient should receive these agents. Although some have advocated the use of amniocentesis to identify the neonate with pulmonary maturity, other nonpulmonary complications of prematurity are still important morbidity factors. Goldenberg et al.<sup>20</sup> reviewed morbidity and mortality data for gestational ages between 22 and 36 weeks. Their conclusions, based on birth weight rather than gestational age, suggest that delaying delivery after 34 weeks of gestation was of little benefit. However, we have found that in the preterm gestation, uncomplicated by other maternal or fetal problems, a delay in delivery from 34 weeks to 36 completed weeks of gestation will decrease the incidence of several neonatal morbidity factors including respiratory distress, patent ductus arteriosus, need for prolonged intensive care, and overall number of hospitalization days. In this institution, we are willing to initiate tocolytic treatment in pregnancies up to 36 completed weeks and continue oral treatment until 37 to 38 weeks of gestation.

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## Management of postdate pregnancy

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Management of the problems associated with pregnancies that extend beyond 294 days of amenorrhea has become increasingly important in obstetrics. This article outlines some of the methods that minimize the risks to the mother, fetus, and neonate in postdate pregnancy. A brief description of the definitions, incidence, and impact of postdate pregnancy is given for a baseline on which to base management decisions. The current management techniques are then given for the following aspects: (1) diagnosis, (2) antepartum surveillance, (3) timing of delivery, and (4) intrapartum management. Finally a synopsis of research areas that may change management is given. (*AM J OBSTET GYNECOL* 1986;154:8-13.)

**Key words:** Postdate pregnancy, prolonged pregnancy, postmaturity

There has not always been widespread acceptance of the fetal risk when pregnancy lasts more than 14 days past the estimated date of confinement. Ballantyne,<sup>1</sup> as early as 1902, described postmaturity as a hazard to the safety of both the infant and the mother. His description included the risks of stillbirth, intrapartum asphyxia, and birth trauma associated with prolonged

pregnancy. In 1952, the detailed description of postmaturity by Clifford<sup>2</sup> defined the risks of uteroplacental insufficiency in postdate pregnancy. The Clifford staging system described increasing degrees of uteroplacental compromise that led to progressive fetal wastage and perinatal mortality. Despite these identifications most American obstetricians debated whether postdate pregnancy was truly a problem while the British and pediatric literature continued to publish on the risks of postterm pregnancy. McClure-Browne<sup>3</sup> presented data from 16,986 pregnancies, which clearly showed that perinatal morbidity increased after 42 weeks, and sug-

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gested that morbidity from routine induction of labor equaled that with nonintervention at that point. Gradually acceptance increased in the United States with the advent of more accurate dating techniques and decreasing morbidity from other perinatal problems. The development of antepartum surveillance, which was shown effective in several well-controlled studies, gave an alternative to routine induction of labor. Today research into cervical ripening agents and improved antepartum testing offer hope that morbidity due to postdate pregnancy can be further reduced.

### **Definitions, incidences, and impact of postdate pregnancy**

Although there have been various definitions of the postdate pregnancy, most authors now use the term to describe pregnancy with a duration that has exceeded 42 weeks (294 days) from the onset of the last menstrual period. Unfortunately such a definition includes patients with uncertain dates. Therefore, a second term, prolonged pregnancy, should be used to describe patients who have pregnancies that are well documented to have exceeded 294 days. Postmaturity should be used to describe the condition of the fetus that is comparable to dysmaturity in the preterm and term neonate. This condition of wasting is thought to be caused by in utero deprivation of nutrients resulting from uteroplacental insufficiency.

When based on menstrual data, approximately 3.5% to 12% of pregnancies will complete 42 weeks of amenorrhea and one fourth (1% to 4% of all pregnancies) will complete 43 weeks.<sup>3,4</sup> Ovulation data have suggested that a number of pregnancies that are postdates by menstrual data are not prolonged pregnancies and may explain part of the variation in incidences reported. Differences in the incidence of postdate pregnancy with respect to parity, maternal age, and race have been debated in the literature. Inaccuracy of dating of pregnancies and irregularity of ovulation could account for the variations seen.

The impact of postdate pregnancy on perinatal mortality has been well studied. Before antepartum testing, the incidence of stillbirth was high. Clifford<sup>2</sup> found that for every postmaturity-associated neonatal death there were five fetal deaths. These stillborn fetuses frequently demonstrated signs of chronic hypoxia and malnutrition, including petechiae of the myocardium and pleura, adrenal hyperplasia, amniotic debris in the lungs, and decreased fat depots. These changes all suggested chronic in utero nutritional and/or respiratory deprivation. In addition, congenital malformations are increased by 50% in postterm pregnancies<sup>4</sup> and account for a significant portion of the published mortality. The impact of these fetuses is decreasing with the advent of ultrasound and better prenatal identification. McClure-

Brown<sup>3</sup> found that without intervention perinatal mortality was 10.5 at 39 to 41 weeks, doubled to 20.0 by 42 weeks, and doubled again to 40.0 by 44 weeks. With the advent of antepartum testing, intrapartum electronic monitoring, and improved neonatal care, these numbers have been considerably reduced.<sup>5-7</sup>

While mortality has been significantly reduced, the associated morbidity continues to affect neonates and mothers. Freeman et al.<sup>5</sup> found that the incidence of cesarean section rose from 13.6% at term to 25.6% in the postterm group. Failed progress of labor as an indication for primary cesarean section was increased 2½ times in postdates patients. There were 10.2% of term infants compared with 25.2% of postterm infants weighing >4000 gm, which perhaps explains this increase in failed progress of labor as an indication for primary cesarean section. An increased cesarean birth rate carries a marked increase in maternal morbidity. Intrapartum fetal distress requiring cesarean section increased from 1.0% to 3.5% in postterm pregnancies.<sup>7</sup>

Clearly the infant with postmaturity syndrome is at increased risk of morbidity and mortality. The undernourished neonate may show temperature instability, metabolic instability, and neurological damage. These fetuses tolerate labor poorly in severe cases. Dysmaturity at any gestational age may produce growth retardation and possible subtle neurological damage.<sup>2,4</sup>

While 80% of postterm infants will not develop postmaturity syndrome, problems may still occur. Callenbach and Hall<sup>8</sup> found that well-nourished postterm infants may be at a higher risk of morbidity and mortality than infants with postmaturity syndrome. This may be explained by the larger infants having longer labors and more traumatic deliveries but also raises concern that the postterm brain may be less able to tolerate asphyxia without long-term damage. The chance for long-term neurological damage also rises with gestational age and is the most costly of all morbidity.<sup>4</sup> Meconium-stained amniotic fluid occurs twice as often in postdate pregnancies than in term pregnancies (25% versus 12%).<sup>7</sup> It is not clear whether this is secondary to the presence of a more dominant vagal system or whether there is an actual increase in intrauterine insults. Regardless of the cause, meconium can cause long- and short-term problems.

### **Diagnosis of prolonged pregnancy**

The diagnosis of prolonged pregnancy requires the accurate determination of gestational age. Unfortunately accurate dating of pregnancy cannot be done in the term and postterm patient. Therefore, if pregnancy dates have not been documented appropriately early in gestation, it is too late to begin when the pregnancy is thought to be postdate. This forces the clinician to determine gestational age before the time when post-



term pregnancy can be anticipated and requires that, when possible, all pregnancies be dated early in gestation. This will not be feasible in all patients since many will arrive late for prenatal care for various reasons and will have to be managed as if they truly had a prolonged pregnancy.

A number of studies have demonstrated the efficiency of history and physical examination in determining gestational age. Anderson et al.<sup>9</sup> showed that a known date of last menstrual period was the best clinical predictor of date of confinement. In fact, prediction of the actual day of delivery in patients with a known date of last menstrual period was not improved with the addition of ultrasonic measurements. In the same study only 71% of patients could recall the exact date of their last menstrual period, 25% gave an approximate date, and in 4% the dates were completely unknown. Besides menstrual data, other information obtained from the patient's history can be important. Basal body temperature data or other ovulatory history data must be considered accurate and used when available. History taking should also screen for factors that may cause delayed ovulation such as previous oligoovulation, ovulation-inducing agents, and recent discontinuance of hormonal contraceptive agents. Quickening, described as the date when the patient feels fetal movement for three consecutive days, has been shown to estimate date of delivery by +17 days and should be used to support other clinical information.

Physical examination should be used to confirm the dates obtained by history. Anderson et al. found that, secondary to menstrual data, the fundus reaching the umbilicus at 20 weeks was the second most sensitive indicator of gestational age. The other parameters of measured fundal height, quickening, and first auscultated fetal heart tones were found to have equal accuracy. Obtaining multiple clinical factors or several fundal height measurements improved the estimate. First-trimester examination of the pregnant uterus is usually helpful except in the obese patient or in the patient with uterine malformations or leiomyomas.

Hormonal methods of estimating gestational age have also been described. They have the advantage of being totally noninvasive and relatively inexpensive. The earliest method described involved serial determinations of serum estriol levels to detect the surge that occurs between 35 and 36 weeks. Placental polypeptides have also been shown to be predictive of gestational age. Human placental lactogen measurements during the first trimester and in combination with human chorionic gonadotropin levels during the second trimester have predicted gestational age. Lagrew et al.<sup>10</sup> measured quantitative levels of human chorionic gonadotropin in patients <60 days' gestation and observed only a 3.5-day mean difference between predicted and actual gestational age. This method is par-

ticularly useful with infertility patients or possible abnormal pregnancies such as ectopic pregnancy or threatened abortion where serum human chorionic gonadotropin determinations are used for other clinical purposes.

Ultrasonic determination of gestational age has become the "gold standard" of backing up history and physical examination information. Since almost any growing structure can be visualized and measured, those with definable anatomic landmarks and the most rapidly changing have been used. During the first trimester gestational sac diameter and crown-rump measurement have been described. Gestational sac measurement was actually one of the first measurements described but sometimes proved inaccurate because of inclusion of the yolk sac. The rapidly growing crown-rump length predicts gestational age within 5 days during the first trimester. At around 12 weeks the fetus begins developing a kyphosis and the method loses accuracy.

By 14 weeks of amenorrhea the fetal biparietal diameter and fetal femur length can be measured consistently. The accuracy of the two methods is well established, with the former being slightly more accurate. The best prediction is obtained before 28 weeks with a range of variation of  $\pm 1.5$  to 2 weeks (95% confidence level) while the fetal skeletal system is rapidly growing. After the twenty-eighth week, as growth of these structures slows down, accuracy declines to  $\pm 3$  to 4 weeks.<sup>11</sup> Correction for head distortion, either flattening or rounding, can be accomplished by measuring head circumference. Abdominal circumference measurement is less accurate than other methods but is important in assessing fetal growth. The best estimate appears to come from obtaining all of these measurements and averaging to adjust for a single improper measurement. Furthermore cross-checking with the cephalic index (ratio of the biparietal diameter to the occipitofrontal diameter) and the ratio of the femur length to the biparietal diameter can be used. Both of these values should fall within  $79\% \pm 8\%$ .

#### Antepartum surveillance

Since not all pregnancies can be appropriately dated and there are no completely successful methods of inducing labor, one must provide appropriate antepartum surveillance. The reason for testing should be to screen for uteroplacental insufficiency and identify the fetus who would benefit from delivery. Testing methods must be reasonably cost effective, acceptable to the patient population, and reliable. Furthermore they should minimize unnecessary intervention, which would result in increased maternal morbidity.

Most authors would agree that testing should begin no later than the start of the forty-second week of amenorrhea. This point in gestation was chosen be-

cause mortality has nearly doubled by this time period and reasonable numbers of patients can be tested. Between the fortieth and forty-second weeks the number of patients drops from roughly 50% to 10% of all pregnancies. Therefore screening prior to 42 weeks must be limited to inexpensive methods that are easy to perform. This includes such methods as fetal movement counting, amniotic fluid volume scanning, and hormonal assays. Patients with abnormal findings by these screening methods should then begin more intensive methods. It has also been suggested that the 40- to 42-week interval may carry some risks and further studies are indicated to see if earlier testing would be cost effective.

There have been several schemes presented for primary surveillance in postdate pregnancy including (1) estriol determination, (2) nonstress testing with a contraction stress test backup, (3) contraction stress testing as primary surveillance, and (4) real-time ultrasound fetal evaluation. Selection of the appropriate scheme involves choosing the system that gives the best results with the available equipment and personnel.

Hormonal evaluation has been mainly used for screening and in large populations where other testing is not feasible. Human placental lactogen will fall with decreasing placental function but the test is prone to false negative and false positive results that make it a poor method of primary surveillance. Estriol determinations can reflect fetal well-being or jeopardy. Most authors feel that estriol monitoring is convenient but the false negative rate is higher. More recently the estriol/creatinine ratio in urine has been used to test fetal status. The data of Khouzami et al.<sup>6</sup> suggested that this was a better measure of fetal well-being than the nonstress test or the contraction stress test. During their series, intervention was done for heart rate test results and not hormonal results; therefore, the intervention role for this method was not established.

The most widely used method of testing is the nonstress test. The test is noninvasive, is simple to perform and interpret, and can be done on relatively inexpensive monitoring equipment. Since there are many benign reasons for nonreactivity the nonreactive nonstress test should be followed with a contraction stress test to prove fetal jeopardy. Unfortunately the antepartum fetal death rate appears to be high when the test is done on a weekly basis.<sup>7</sup> Since loss of reactivity due to uteroplacental insufficiency is a rather late sign,<sup>12</sup> it is not surprising that testing done 1 week beforehand would fail to predict fetal death. Therefore nonstress testing, if used, should be performed on a twice-weekly basis and it should be realized that any decelerations seen on the nonstress test require further follow-up regardless of the presence of reactivity.

Contraction stress testing is based on the principle that the fetus is stressed during a uterine contraction

by the cessation of blood flow to the uteroplacental bed. Fetuses with lowered reserve from chronic uteroplacental insufficiency will develop late decelerations and consequently have a positive test. This is an earlier sign of uteroplacental insufficiency than loss of reactivity. Thus the contraction stress test on a theoretical basis should identify fetuses in early jeopardy. The test is more time consuming and requires more equipment and personnel training than the nonstress test but clinical studies have confirmed the low false negative rate. Freeman et al.<sup>5</sup> reported on 679 patients evaluated with contraction stress tests for postdatism without a single perinatal death. Furthermore intervention for an abnormal test result was only 5.4%. With development of the nipple stimulation contraction stress test the test has become less time consuming and easier to perform.

The advent of ultrasound has given a new view of the unborn and ultrasonic fetal well-being studies have resulted. Eden et al.<sup>7</sup> published results of the combination of twice-weekly nonstress tests and weekly real-time ultrasound scanning that compared favorably with the aforementioned contraction stress test results. There were 109 patients evaluated with this method and 23.9% had intervention for either decreased amniotic fluid volume, fetal heart rate decelerations on nonstress test, or a nonreactive nonstress test with absence of fetal movement or breathing. The numbers are somewhat small and the higher intervention rate may have explained the increased incidence of cesarean section required in the nonstress test-real-time ultrasound group when compared with patients evaluated by contraction stress tests. Manning et al.<sup>13</sup> reported results with the biophysical profile in 1184 high-risk patients. One hundred seventy-eight of these were postdate pregnancies and there was one fetal death at 42 weeks, which occurred 5 days after a 10/10 biophysical profile. One important benefit from ultrasonic evaluation is the increased detection of fetal anomalies. Further research in this area should give the efficacy and necessary interval for testing when ultrasonic tests of well-being are used.

### Timing of delivery

Many authors have suggested the routine induction of labor at 42 weeks. While McClure-Brown<sup>3</sup> originally advocated routine induction of labor, several studies have shown that there is no improvement in perinatal outcome when routine induction is compared with nonintervention.<sup>4</sup> The reason for failure of improved outcome by routine induction probably can be explained by the following: (1) the increased morbidity with no benefit given to postdate patients with delayed ovulation or poor dates who do not have true prolonged pregnancies by ovulation-indicated gestational age; (2) the fact that most fetuses, even despite prolonged pregnancies, are not compromised; (3) failure of induction



with its inherent maternal morbidity, and (4) prolonged induction with lengthy labor that depletes fetal reserve in a fetus that would have tolerated a labor of normal length.

Timing of delivery should be made on the basis of cervical status. Most authors agree that patients with well-documented dates and ripe cervixes should undergo induction of labor. This circumvents the expense of testing. Unfortunately this will only include a minority of postdate patients. Harris et al.<sup>15</sup> found that in a group of well-documented 42-week pregnancies the mean cervical Bishop score was 3.6 and only 8.2% had a score of  $\geq 7$ . This study also points out the fallacy of redating in the postdate patient because the cervix is unripe.

When the cervix is unripe the decision of delivery is made on the basis of antepartum testing. Our current recommendation is to use contraction stress testing for primary surveillance. Our protocol recommends starting weekly contraction stress tests at the beginning of the forty-second week. Negative tests are repeated at weekly intervals until delivery or until cervical ripeness is demonstrated. Equivocal tests, whether due to hyperstimulation or suspicious results, are repeated within 24 hours. Patients with positive tests, with reactivity, are allowed a trial of labor since only one half will require cesarean section for fetal distress. All patients with positive nonreactive tests are delivered by cesarean section since virtually all will have fetal distress in labor.

For screening purposes quantitative amniotic fluid volume can be added to this testing scheme, with weekly estimation of amniotic fluid volume beginning at 41 weeks. Patients with marginally low fluid volume or oligohydramnios should undergo induction of labor. If routine screening for amniotic fluid volume is not performed it should be considered in any patients demonstrating variable decelerations during fetal heart rate testing, as the decelerations could be secondary to cord compression from oligohydramnios.

#### **Intrapartum management**

Proper antepartum management is important in postdate pregnancy but does not ensure good outcome without continuance of proper management in the intrapartum period. Antepartum testing screens for stillbirth and advanced uteroplacental insufficiency. Nonetheless some fetuses will have enough fetal reserve for normal antepartum testing results but will exceed that reserve with the greater stress of labor. Most studies have found there will be an increased incidence of fetal distress even in patients with normal antepartum test results. Furthermore there is some suggestion that the decreased fluid in postterm patients may lead to increased cord compression during labor, which may fur-

ther compromise the fetus. Meconium also becomes a frequent problem in the postdate pregnancy because all fetuses of that gestational age will have readily activated vagal systems and the decreased level of amniotic fluid fails to dilute meconium that is passed and thus the consistency of the meconium is thick and tenacious. Finally while the mean birth weight of infants born after term does not seem to rise, there is a spectrum of growth. Some fetuses will show evidence of decreased growth or even weight loss and be designated postmature. In some cases the placenta will have only partial failure and the weight will change only slightly after term. Some placentas will continue to provide nourishment and the fetus will continue to grow, leading to macrosomia. These macrosomic fetuses will be more likely to have prolonged labors, failure to progress, and birth trauma.

The postdate fetus is clearly at higher risk to develop fetal distress and therefore should be electronically monitored from the onset of labor to delivery.<sup>5</sup> A special concern for bizarre intrapartum fetal heart rate patterns should be noted. The fetus that has neurological dysfunction due to chronic asphyxia or other causes may not display classic signs of fetal distress. The clinician should be aware that a smooth baseline, blunted variable decelerations, or a wandering baseline may be a sign of significant fetal central nervous system dysfunction or previous damage. A quick ultrasound evaluation may be warranted in such patients since these patterns are also seen with major neurological malformations such as anencephaly. The previously damaged or anomalous fetus may have a normal acid-base status as reflected by scalp sampling but show a flat, blunted fetal heart rate pattern. Thus, if one can document a normal fetal scalp blood pH in such patients, cesarean section will probably not benefit them.

The proper management of meconium is important because of the increased incidence and the fact that these infants have an increased chance of fetal distress and low Apgar scores. The physician and nursing staff must be careful to identify the patient with meconium-stained amniotic fluid and to have equipment and personnel ready for delivery. This may be somewhat difficult because the fetus with diminished fluid may be associated with only small amounts of vaginal discharge before delivery. Delee suctioning on the perineum at vaginal delivery or on the abdomen for cesarean deliveries has become standard care by most institutions. This involves aggressive suctioning of the nasopharynx and posterior oropharynx before delivery of the shoulders. This should be combined with proper endotracheal suctioning for meconium beyond the vocal cords after delivery. This will practically eliminate cases of severe meconium aspiration syndrome although, because of rare instances of intrauterine aspiration, not

all cases of meconium aspiration can be prevented. Finally special attention should be directed to recording a detailed description of the management of meconium in the delivery notations and consideration should be given to obtaining umbilical cord blood gas measurements. The latter will document neonatal status before the suctioning process since Apgar scores may be artificially lowered by suppression of respirations during suctioning.

Finally proper management of the 25% of infants that will weigh >4000 gm is important. Because of the problem of macrosomia associated with prolonged pregnancy, intrapartum management of dystocia becomes important. The anticipation of possible shoulder dystocia may be helpful, although the problem is not as great as with the diabetic macrosomic infant. Benedetti and Gabbe<sup>16</sup> showed that the combination of midpelvic delivery and macrosomia was associated with a significantly higher incidence of shoulder dystocia than when delivery was spontaneous or by low forceps. Therefore avoidance of midpelvic vaginal delivery is recommended when macrosomia is suspected.

#### Future developments

There are a number of developments that may improve outcome and management of postdate pregnancies. While more accurate methods of gestational age assessment are possible, there are no experimental methods that appear to have tremendous advantage over current methods. Research continues in antepartum surveillance, with testing of new methods and comparison of current techniques to choose the best method. Such studies are difficult because of the large populations required to demonstrate differences. Effective cervical ripening agents, such as prostaglandin gel or relaxin, may allow easier inductions. Clinical trials continue with these agents to demonstrate efficacy and safety. Finally, during labor, the practice of intrauterine saline infusion in patients with ruptured membranes may decrease intrapartum cord compression and allow fetuses to tolerate longer labors.

#### Comment

In summary, a number of management suggestions can be given for dealing with postdate pregnancy.

1. Attempts should be made to determine the estimated date of confinement in all pregnancies before the third trimester and "redating" a pregnancy should not be done in term and postterm pregnancies.

2. Antepartum surveillance should be performed in all pregnancies that might have reached 42 weeks of amenorrhea. We would suggest weekly contraction stress tests for surveillance.

3. The timing of delivery should be made on the basis of cervical status. Patients with ripe cervixes and

prolonged pregnancy should undergo induction of labor. Otherwise abnormal results of antepartum testing, well-documented oligohydramnios, or reaching of the forty-third week of amenorrhea in a pregnancy with accurate dates should be considered indications for delivery.

4. During the intrapartum period special attention should be paid to careful electronic fetal monitoring, proper management and documentation of the management of meconium-stained amniotic fluid, and avoidance of midpelvic vaginal delivery for possibly macrosomic infants.

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# Caffeine consumption during pregnancy and association with late spontaneous abortion

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In a prospective cohort study, 3135 pregnant women were followed to evaluate the association of caffeine consumption during pregnancy with late first- and second-trimester spontaneous abortion. Almost 80% of pregnant women used some caffeine; among users the average daily intake was 99.3 mg from all sources. Sources of caffeine were nonherbal tea (used by 49.4% of women), coffee (41.2%), colas (35.0%), and drugs (6.6%). In all, 28% of pregnant women consumed  $\geq 151$  mg of caffeine daily, and these "moderate-to-heavy" caffeine users were significantly more likely to experience late first- or second-trimester spontaneous abortion when compared with nonusers and light users (0 to 150 mg). Demographic characteristics, reproductive and medical history, contraceptive use, and smoking and drinking habits were taken into consideration. The adjusted relative risk of miscarriage after moderate-to-heavy caffeine consumption was 1.73 ( $p = 0.03$ ). Light caffeine use (1 to 150 mg daily) was associated with increased risk for spontaneous abortion only among women who aborted in their last pregnancy ( $RR = 4.18$ ,  $p = 0.04$ ). Replicative studies are necessary before the association of caffeine with spontaneous abortion can be confirmed. (AM J OBSTET GYNECOL 1986;154:14-20.)

**Key words:** Caffeine, pregnancy, spontaneous abortion

Caffeine is listed in the Code of Federal Regulations as a multipurpose food substance that is generally recognized as safe. It can be found in cold tablets, allergy or analgesic preparations (15 to 64 mg/U), appetite suppressants (50 to 200 mg/U), and stimulants (100 to 200 mg/U). As a beverage, caffeine is ingested daily by a large segment of the American population as coffee (29 to 176 mg/cup), tea (8 to 107 mg/cup), cocoa (5 to 10 mg/cup), solid milk chocolate (6 mg/ounce), and cola beverages (32 to 65 mg/12 oz).<sup>1</sup> Less is known about caffeine consumption during pregnancy.

In animals several studies have suggested a possible link of caffeine to birth defects, fetal resorption, and decreases in fetal weight.<sup>2</sup> Although the extrapolation of findings from animal studies to humans is not straightforward, the U.S. Food and Drug Administra-

tion cautions pregnant women to avoid caffeine or to use it sparingly.

Caffeine is readily absorbed from the gastrointestinal tract and is rapidly distributed throughout all tissues as a function of water content. It also crosses the placenta.<sup>3</sup> In pregnant women the plasma half-life has been observed to increase to 10.5 hours as compared to 2.5 to 4.5 hours in healthy adults.<sup>4</sup> The plasma half-life in the newborn infant ranges from 32 to 149 hours.<sup>5</sup>

Potential effects of caffeine on fetal development may follow prolonged accumulation of caffeine in pregnant women and passage to the fetus which lacks enzymes necessary for the metabolism of caffeine until several days after birth.<sup>5</sup> Additionally, caffeine is known to increase adenosine-3':5'-cyclic monophosphate in cells and may interfere with fetal cell growth. Caffeine may act directly on nucleic acids,<sup>6</sup> since it is structurally similar to adenine and guanine, and result in chromosome aberrations. Another possible mechanism of caffeine action is by the increase of catecholamines,<sup>7</sup> which may restrict uteroplacental circulation through vasoconstriction and result in fetal hypoxia. Fetal deaths, decreases of fetal weight, fetal malformations, and shortened gestational age can follow fetal hypoxia.

Existing human studies provide incomplete and conflicting results of the effect of caffeine consumed dur-

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**Table I.** Daily consumption of coffee, tea, and colas during pregnancy in the study population

Frequency of use	Coffee		Tea		Colas	
	n	%	n	%	n	%
Nonusers	1843	58.8	1586	50.6	2037	65.0
1-3 cups/wk	422	13.5	594	19.0	617	20.0
4-6 cups/wk	154	4.9	209	6.7	194	6.2
1-2 cups/day	553	17.6	585	18.7	219	7.0
3-4 cups/day	139	4.4	129	4.1	50	1.6
5-10 cups/day	22	0.7	25	0.8	13	0.4
10+ cups/day	2	0.1	7	0.2	5	0.2
Total	3135	100.0	3135	100.0	3135	100.0

ing pregnancy on the risk for birth defects,<sup>8</sup> preterm deliveries,<sup>9-11</sup> and low birth weight.<sup>10,12</sup> Only one study<sup>9</sup> reported a higher rate of spontaneous abortion among 16 women whose caffeine consumption during pregnancy was high (>600 mg). This study almost certainly used an unrepresentative sample of patients, since they had an unusually high stillbirth rate of 12.7%. Potential confounding variables were not controlled in the analysis and risks of moderate caffeine intake were not studied.

The objectives of the present study are (1) to provide a detailed description of caffeine exposure in a large population of pregnant women and (2) to investigate the relationship between caffeine consumption during pregnancy and spontaneous abortion.

### Material and methods

Candidates for the study were all women who planned to be delivered at Yale New Haven Hospital and who sought care from 29 private physicians and health maintenance organizations in the New Haven area between May, 1980, and March, 1982. Each woman was interviewed during pregnancy by a trained interviewer with use of a standardized schedule. Information obtained included demographic characteristics, previous medical and obstetric history, smoking and drinking habits during pregnancy, and occupational exposure. Caffeine consumption was calculated from caffeinated coffee, tea, and colas (including all caffeinated soft drinks) with use of, respectively, 107 mg/serving, 34 mg/serving, and 47 mg/serving.<sup>1</sup> The names, numbers of days, times per day, and the weeks in gestation when prescription and nonprescription drugs were used during the first trimester of pregnancy were ascertained to identify those containing caffeine. Drug recall was enhanced by probing for 22 specific conditions (e.g., for control of nausea, for fluid retention, for diet or weight loss).

Spontaneous abortion was defined as nondeliberate interruption of an intrauterine pregnancy of <28 weeks' gestation in which the fetus was dead when expelled. Gestational age at spontaneous abortion was

computed based on date of conception and the date of abortion. Date of conception was based on last menstrual period or physician's estimated due date for respondents when the last period was uncertain. Spontaneous abortions (n = 68) were identified from medical records and follow-up contacts with obstetricians and respondents. All miscarriages in the study occurred after interview and between 8 and 26 weeks' gestation.

A total of 4073 women were eligible for the study. Women were considered ineligible for interview if they (1) were not pregnant at the time of interview, including women who spontaneously aborted or who were delivered before interview; (2) did not plan to deliver at our hospital; (3) demonstrated poor English comprehension; and (4) were familiar with the study. Of the eligible women, 473 refused to be interviewed, and 255 could not be reached to arrange an interview. For five subjects the interviewer considered the interview to be invalid. Interviews were completed on 3340 (82.0%) of eligible women. The pregnancy outcome of a further 86 women could not be ascertained, and 95 women were excluded because their gestational age at interview was >28 weeks and they were no longer at risk for spontaneous abortion. Some detail of caffeine consumption was lacking in 24 women, so we could not compute their average daily caffeine intake. The analysis uses 3135 subjects.

### Results

**Frequency of caffeine consumption during pregnancy.** Nonherbal tea was the most frequently used caffeinated beverage (drunk by 49.4% of all respondents) followed by coffee (41.2%) and colas (35.0%) (Table I). Neither coffee nor tea drinking was reported by 30.7% of respondents and 21.9% did not drink coffee, tea, or colas. The average servings daily were tea, 0.6; coffee, 0.6; and colas, 0.3. The frequency for drinking three or more servings per day of coffee, tea, and colas was 5.2%, 5.1% and 2.2%, respectively.

Overall, 6.6% of women used a caffeine-containing prescription or nonprescription drug at least once in the first trimester. Most of these women (95.1%) used



**Table II.** Source and amount of average daily caffeine consumption by type of consumer

Source	Coffee used		No coffee used		No coffee or tea used		No coffee, tea, or colas used	
	(n = 1292)		(n = 1843)		(n = 963)		(n = 635)	
	mg	%	mg	%	mg	%	mg	%
Coffee	143.7	77.6	—	—	—	—	—	—
Tea	20.1	10.9	21.2	54.2	—	—	—	—
Colas	16.2	8.7	13.4	34.4	12.5	77.3	—	—
Drugs	5.1	2.8	4.5	11.5	3.7	22.7	3.9	100.0
Total	185.1	100.0	39.1	100.0	16.2	100.0	3.9	100.0

only one caffeine-containing drug. Excedrin (64.8 mg of caffeine per unit) was the most frequently used caffeine-containing drug (by 23.7% of caffeine-drug users), followed by Anacin (32.5 mg of caffeine, 19.1% of users), Dristan (16.2 mg, 14.9% of users), Fiorinal (40 mg, 8.8% of users), and Dexatrim and Dietac (200 mg, 6.5% of users). However, these drugs were used sporadically; 54.6% used them for a period of 1 to 3 days and 23.7% for 4 to 14 days.

In all, 652 women (20.8%) used no caffeine in pregnancy, 1604 (51.2%) were light (1 to 150 mg daily) caffeine users, and 879 (28.0%) were heavy ( $\geq 151$  mg daily) caffeine users. The source of caffeine for different consumers is shown in Table II.

**Characteristics of women who used caffeine during pregnancy.** Daily caffeine consumption is categorized as none (0 mg), light (1 to 150 mg), and moderate-to-heavy ( $\geq 151$  mg). Table III summarizes the demographic characteristics of women who used and did not use caffeine during pregnancy. Moderate-to-heavy users are significantly more likely to be over 30 years of age, be white, have  $\leq 12$  years of education, be relatively heavy users of alcohol (daily consumption of absolute alcohol of  $>0.25$  ounces), be cigarette smokers, use marijuana during pregnancy, be in households whose head is a skilled or unskilled worker, and be interviewed beyond 12 weeks' gestation. Mothers not using any drugs during the first trimester, and Jewish women are significantly less likely to be caffeine users. Moderate-to-heavy caffeine users are significantly more likely to have higher gravidity and parity, be women with a history of spontaneous abortion, and be less likely to have a history of gynecologic surgery or any gynecologic condition.

Menarcheal age, total numbers of prior induced abortions, interval from last pregnancy and history of any infertility treatment 12 months before pregnancy are not statistically different among the three caffeine exposure groups (Table IV). All contraceptive methods used during the month before conception, at the time of conception, or 1 month after conception were similar in the three caffeine use groups.

**Maternal characteristics associated with spontaneous abortion.** Women who spontaneously aborted were significantly more likely to be over 30 years of age. Jewish women had a higher spontaneous abortion rate, but this increase is not statistically significant ( $p = 0.09$  for Jewish versus non-Jewish). Marital status, education, religion, ethnic background, number of drugs used in the first trimester, medical conditions 12 months before pregnancy, any injury during pregnancy, diethylstilbestrol used by respondents' mother, cigarette smoking, alcohol, and marijuana use during pregnancy were not markedly different in the group with spontaneous abortions and in the group without them. As expected, women interviewed early in pregnancy were significantly more likely to subsequently abort.

Spontaneous abortion rates were significantly higher in women who became pregnant (with the index pregnancy) within 6 months of the last pregnancy ( $p < 0.001$ ) and with a history of gynecologic surgery ( $p = 0.03$ ). Menarcheal age, gravidity, parity, history of any previous spontaneous abortion or induced abortion, any infertility treatment 12 months before the index pregnancy, and termination of the last pregnancy with a spontaneous abortion were not significantly different between the two groups. The use of pill, intrauterine device, foam, cream, jelly, suppository, diaphragm, rhythm, condom, and withdrawal were not significantly associated with spontaneous abortion.

**Frequency of spontaneous abortion and crude association with caffeine used.** In all, 2.2% of the study subjects spontaneously aborted after the interview. The rate for non-caffeine users was 1.8%; for light caffeine users, 1.8%; and for moderate-to-heavy caffeine users, 3.1% ( $p = 0.096$ ). Because of the similar spontaneous abortion rate in the no caffeine and light caffeine users, this group was combined (0 to 150 mg). The crude relative risk for spontaneous abortion of moderate-to-heavy use ( $>150$  mg) was 1.69 (95% confidence intervals = 1.04 and 2.71,  $p = 0.030$ ).

**Multivariate analysis of association between caffeine consumption during pregnancy and sponta-**

**Table III.** Maternal characteristics of caffeine users during pregnancy by average daily caffeine use

Characteristics	n	Average daily caffeine exposure (mg)			Significance tests
		0	1-150	151+	
Age					
≤30 years	2226	21.7	52.9	25.4	$\chi^2_3 = 26.94$ p = 0.0001
>30 years	909	18.6	46.9	34.5	
Marital status					
Currently married	2643	20.7	51.4	27.9	$\chi^2 = 0.88$ p = 0.64
Not currently married	192	21.9	47.9	30.2	
Religion					
Catholic	1606	15.8	51.1	33.1	$\chi^2_3 = 76.63$ p = 0.0001
Protestant	1019	24.7	52.0	23.3	
Jewish	210	30.5	51.9	17.6	
None	211	28.4	45.5	26.1	
Other	67	26.9	50.8	22.4	
Education (yr)					
<12	148	16.2	45.3	38.5	$\chi^2_3 = 43.06$ p = 0.0001
12	785	15.5	51.7	32.7	
13-16	1575	21.4	52.1	26.5	
17+	627	27.0	49.4	23.6	
Ethnic background					
White	2873	29.2	51.5	28.4	$\chi^2_2 = 12.13$ p = 0.002
Nonwhite	257	20.1	47.1	23.7	
Occupation of head of household					
Higher executive, lesser professional	1090	23.5	52.1	24.2	$\chi^2_2 = 22.30$ p = 0.0002
Administrative personnel, technician	1080	21.7	49.4	29.0	
Skilled or unskilled labor	954	16.6	52.3	31.1	
Gestational age at interview (wk)					
≤12	1875	20.6	52.8	26.6	$\chi^2_2 = 6.13$ p = 0.047
>12	1260	21.0	48.7	30.2	
No. of drugs used*					
0	968	25.7	48.1	26.1	$\chi^2_2 = 22.17$ p = 0.0002
1	988	18.5	53.9	27.6	
2+	1178	18.7	51.4	29.9	
Medical conditions 12 months before pregnancy†					
No	2776	21.1	51.5	27.5	$\chi^2_2 = 4.35$ p = 0.11
Yes	359	18.7	48.8	32.6	
Injury during pregnancy					
No	2917	20.7	51.3	28.0	$\chi^2_2 = 0.63$ p = 0.73
Yes	204	22.6	48.5	28.9	
Mothers exposed to diethylstilbestrol					
No	2590	21.2	51.2	27.6	$\chi^2_2 = 6.18$ p = 0.19
Yes	29	31.0	48.3	20.7	
Uncertain	481	17.9	51.9	31.2	
Alcohol use during pregnancy					
None	804	27.2	51.4	21.4	$\chi^2_2 = 70.29$ p = 0.0001
≤0.25 of absolute alcohol	2088	19.1	52.4	28.5	
>0.25 of absolute alcohol	243	14.0	40.3	45.7	
No. of cigarettes smoked per day during pregnancy					
0	2259	24.1	54.0	21.9	$\chi^2_2 = 195.57$ p = 0.0001
1-10	433	14.3	49.0	36.7	
11+	405	9.1	38.0	52.8	
Marijuana use during pregnancy					
No	2911	21.3	51.3	27.5	$\chi^2_2 = 9.68$ p = 0.008
Yes	222	14.9	49.1	36.0	

\*Prescription and nonprescription drugs were included.

†Heart or circulation problems, kidney problems, high blood pressure, sickle cell anemia, or other medical conditions.

**neous abortion.** Variables associated with both caffeine exposure and miscarriage at the  $p < 0.10$  level of significance were considered potential confounding factors. These were gestational age at interview, maternal age, prior gynecologic surgery, member of Jewish religion, and last pregnancy ending with a spontaneous

abortion. Several classifications of average daily caffeine consumption were used to test for an association with spontaneous abortion. First daily caffeine consumption was trichotomized as none, light (1 to 150 mg), and moderate-to-heavy ( $\geq 151$  mg), and a multiple logistic regression model was fit to the data. This pro-



Table IV. Maternal obstetric history by levels of caffeine exposure and significance tests

Obstetric history	n	Levels of caffeine exposure (mg)			Significance tests
		0	1-150	151+	
Menarcheal age (yr)					
<12	684	21.1	48.4	30.7	$\chi^2_1 = 4.59$
12-13	1796	20.4	52.6	26.8	$p = 0.35$
14+	625	21.8	50.2	27.8	
Gravidity					
1	946	24.7	52.9	22.4	$\chi^2_1 = 40.38$
2	1113	20.5	52.6	27.0	$p = 0.0001$
3	1075	17.7	48.2	34.1	
Parity					
≤1	2574	21.8	52.3	26.0	$\chi^2_2 = 32.55$
2+	561	16.2	46.2	37.6	$p = 0.0001$
Previous spontaneous abortion					
0	1618	18.4	52.0	29.6	$\chi^2_1 = 16.44$
1	445	19.6	49.7	30.8	$p = 0.0025$
2+	126	26.2	33.3	40.5	
Previous induced abortion					
0	1572	17.7	51.2	31.1	
1	492	22.8	48.1	29.1	$\chi^2_1 = 7.30$
2+	125	22.4	49.6	28.0	$p = 0.13$
Spontaneous abortion in last pregnancy					
Never pregnant	943	24.8	52.8	22.4	
No	1843	18.3	51.5	30.2	$\chi^2_1 = 29.92$
Yes	345	23.2	44.6	32.2	$p = 0.0001$
Induced abortion in last pregnancy					
Never pregnant	943	24.8	52.8	22.4	
No	1803	17.8	50.8	31.4	$\chi^2_1 = 38.07$
Yes	386	25.1	48.7	26.2	$p = 0.0001$
Interval from last pregnancy					
≤6 months	199	22.1	48.2	29.7	$\chi^2_2 = 1.36$
>6 months	1950	18.7	50.8	30.5	$p = 0.50$
Gynecologic condition 12 months before pregnancy*					
No	2340	19.4	50.9	29.6	$\chi^2_2 = 16.40$
Yes	795	24.8	51.8	23.4	$p = 0.0003$
Infertility treatment 12 months before pregnancy					
No	2957	20.5	51.2	28.4	$\chi^2_2 = 4.94$
Yes	178	26.4	51.1	22.5	$p = 0.08$
Gynecologic surgery†					
No	2333	19.7	52.7	27.6	$\chi^2_2 = 10.47$
Yes	802	24.1	46.6	29.3	$p = 0.005$

\*Vaginal infections, venereal disease, or pelvic inflammatory disease.

†Caesarean section, induced abortion, or dilatation and curettage given as an abortion procedure are not included.

vided adjusted relative risks that take into account other confounding factors. Moderate-to-heavy use was associated with a twofold increased risk of spontaneous abortion (relative risk = 1.95,  $p = 0.07$ ). Light caffeine use was not by itself associated with an increased rate of spontaneous abortion except among women who also had a history of spontaneous abortion in their last pregnancy. These women had a fourfold increase in spontaneous abortion (relative risk = 4.18,  $p = 0.04$ ).

Next we dichotomized the sample's caffeine use as none and light (0 to 150 mg) and moderate-to-heavy ( $\geq 151$  mg). The multiple logistic regression analysis for this classification of caffeine is shown in Table V. Moderate-to-heavy caffeine use was significantly associated with an increased risk of spontaneous abortion (relative risk = 1.73,  $p = 0.03$ ), as was maternal age over 30 and the methodologic variable—gestational age at interview in the first trimester.

A third multiple logistic regression analysis was used to examine the effect of increasing daily caffeine consumption in 50 mg increments on risk for spontaneous abortion. There was no significant elevation of the adjusted relative risks for caffeine at 150 mg per day or less. At levels  $>150$  mg per day there was a marked increase in the adjusted relative risk for miscarriage, but caffeine consumption of  $>200$  mg did not further increase risk.

An attempt was made to identify the particular source of caffeine that might be associated with spontaneous abortion. However, the number of women in each category is frequently too small to detect other than large effects. Those whose caffeine source was from coffee alone had an increased risk of miscarriage over those whose source was tea alone or colas alone (cRR of 2.0, 1.1, and 1.3, respectively); however, these associations are not statistically significant.

# Comment

Before the results of this investigation are discussed, some limitations need to be noted. First, although the study has a relatively high response rate of 82% of eligible patients, selection bias may occur if women elect to participate based on their caffeine consumption. However, the study was introduced to women as a study of "factors influencing the health of women during pregnancy and their newborn infants" and caffeine was not specifically mentioned. It is unlikely, therefore, that women selectively participated in the study based on their caffeine consumption. Second, the results of the study are limited to only late first- and second-trimester miscarriage. This study therefore cannot evaluate the risk of caffeine consumption during pregnancy on early first-trimester spontaneous abortions. Third, the results are limited to patients seeking their antenatal care at private obstetrical practices and health maintenance organizations. Fourth, although only 86 women (2.7%) were lost to follow-up after interviewing, they could bias the result if these women had a differential rate of spontaneous abortion and caffeine use. However, attrition loss is unlikely to be a major problem, since the average daily caffeine consumption and demographic characteristics of mothers lost to follow-up were not different from mothers who stayed in the study. Fifth, a number of potential confounding variables, for example, maternal illness during the index pregnancy, are not included in the study. Mothers who have some illness, that is, gastrointestinal or cardiovascular problems that may predispose them to spontaneously abort might be advised to avoid or limit caffeine consumption. Such a bias would spuriously reduce the risk estimates for caffeine. In the present prospective study, recall bias could not occur because women were interviewed before knowing their pregnancy outcomes. Sixth, information concerning caffeine consumption is ascertained at the time of interview and cannot necessarily be assumed to remain constant throughout the entire pregnancy. However, a retrospective study at the same hospital<sup>11</sup> found that average coffee and tea consumption for the first, second, and third trimesters of pregnancy are almost the same.

Our estimate that 2.2% of pregnancies end in late first- and second-trimester spontaneous abortion in the study may appear somewhat low. However, if the early spontaneous abortions before interview are included, the rate of 7.2% for spontaneous abortion is not low for a prospective study; those reviewed by Kline and Stein<sup>13</sup> ranged from 3.6% to 9.7%. The finding that 79.2% of the study population was exposed to some caffeine is comparable to the estimate of 74% from the GRAS Survey,<sup>14</sup> which did not include caffeine from drugs.

The proportions of those who drink coffee (41.2%)

**Table V.** Adjusted relative risks and the significance levels of moderately heavy caffeine users and all potential confounding variables

Factors	Adjusted relative risks	p value
Intercept	0.01	0.00
Moderately heavy caffeine users	1.73	0.03
Maternal age, 31+ years	1.72	0.03
Gestational age at interview ≤12 weeks	2.53	0.002
Jewish	1.82	0.12
Prior gynecologic surgery	1.43	0.19
Spontaneous abortion in last pregnancy	1.45	0.29

or tea (49.4%) are somewhat lower than in one previous study but similar to others.<sup>10,11</sup> Comparison of the average daily servings for coffee and tea of the earlier Yale study<sup>11</sup> with the present one suggests pregnant women may have reduced coffee and tea consumption rather than having stopped completely (average daily servings in 1980 to 82 of 0.6 for coffee and 0.6 for tea versus 1.1 for coffee and 1.0 for tea in 1977). It is unlikely that the average daily caffeine intake reported in this study is under reported.

Overall, 6.6% of the study population used at least one caffeine-containing drug during the first trimester of pregnancy. The figure is comparable to that of 10.7% use by pregnant women in the first 4 months' gestation as reported in the Collaborative Perinatal Project,<sup>15</sup> which collected data between 1959 and 1965.

The finding that daily caffeine exposure at the 1 to 150 mg level did not significantly increase the risk for spontaneous abortion whereas it did for the >150 mg level may be interpreted in at least three ways. First, caffeine may have a threshold effect around 150 mg. Ingestion of daily caffeine of <150 mg may not sufficiently affect the fetus to cause fetal death through interference with cell division, cell growth, or uteroplacental circulation. Second, smoking increases demethylation of caffeine in humans,<sup>16</sup> which may reduce the plasma half-life of caffeine and increase its rate of elimination. In this study, smokers are significantly more likely to be caffeine users. Therefore, the effect of caffeine at 1 to 150 mg/day on spontaneous abortion in smokers may be diminished. Third, fetal effects may come from coffee consumption rather than caffeine per se. In this study, coffee is a major source of caffeine in moderate-to-heavy caffeine users (72%) but not in light caffeine users (26%). Benzo (α) pyrene and chlorogenic acid, found in roast coffee,<sup>17</sup> may increase risk for spontaneous abortion. Both caffeinated and decaffeinated instant and roast coffee, but not tea or cocoa, have recently been found to contain an opiate receptor antagonist with binding activity clearly separable from



caffeine.<sup>18</sup> The hypothesis that some component of coffee other than caffeine may be associated with spontaneous abortion should be tested in future research.

Women who have experienced spontaneous abortion in their last pregnancy are known to be at increased risk for subsequent spontaneous abortion. In these women light caffeine use may further increase their susceptibility. This must be considered a preliminary observation, however, since prior miscarriage did not further significantly increase spontaneous abortion risks among moderate-to-heavy caffeine users.

This is the first population-based study to report an association of caffeine with an increased risk of late first- and second-trimester spontaneous abortion. Although the effect of caffeine is consistent in all the statistical models we used, not all analyses achieved conventional statistical significance. Thus, additional studies are needed before the association of caffeine with spontaneous abortion can be more definitively evaluated.

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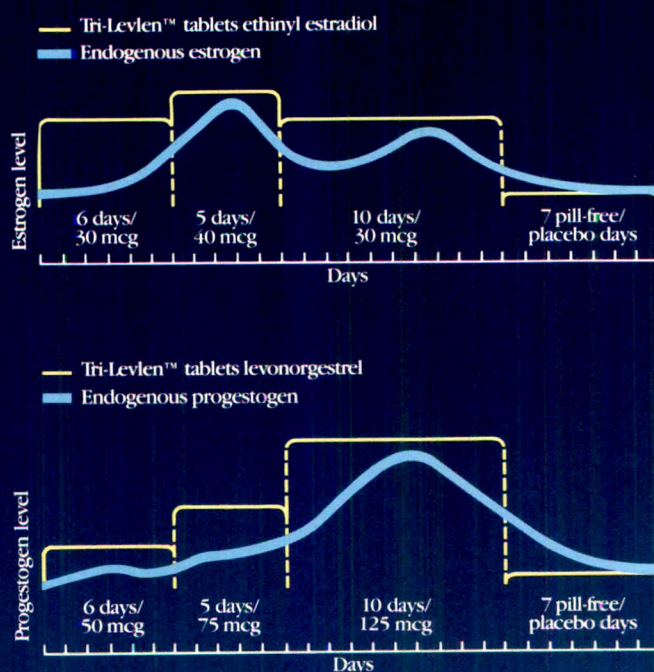
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# Herpes simplex virus testing of an obstetric population with an antigen enzyme-linked immunosorbent assay

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Commercial herpes simplex virus antigen enzyme-linked immunosorbent assay kits were compared to conventional culture for herpes simplex virus with 3237 genital specimens from obstetric patients. These rapid enzyme-linked immunosorbent assay tests had a sensitivity of 34.3% and specificity of 98.1% with primarily cervical specimens from asymptomatic patients. Specimens from vulvar swabs had higher positive rates than those from cervical swabs from the same patient, whether symptomatic or asymptomatic with both culture and enzyme-linked immunosorbent assay. Fifty-six women had enzyme-linked immunosorbent assay false positive tests; use of enzyme-linked immunosorbent assay testing alone could have resulted in 1.7% unnecessary cesarean deliveries. Six cases of neonatal herpes simplex virus infection were identified; two of the mothers had negative cervical cultures the week of delivery preceded by positive vulvar cultures. (AM J OBSTET GYNECOL 1986;154:21-8.)

**Key words:** Herpes simplex virus, enzyme-linked immunosorbent assay, tissue culture, antigen test, obstetric figure

The incidence of both genital and neonatal herpes simplex virus infections has increased ninefold between 1966 and 1982 according to published reports.<sup>1,2</sup> Since herpes simplex virus has the potential of causing fatal infections or permanent neurologic sequelae in neonates, abdominal delivery has been recommended for women with either genital lesions or herpes simplex virus detected near delivery to reduce the incidence of neonatal disease.<sup>3,4</sup>

The American Academy of Pediatrics and others have recommended weekly laboratory herpes simplex virus tests of the cervix of women with a history of genital herpes during the last 6 weeks of pregnancy; vaginal delivery is recommended only for women with two successive negative herpes simplex virus reports.<sup>3</sup> Binkin et al.<sup>1</sup> have recently analyzed these current recommendations, estimating that weekly genital herpes simplex virus testing will prevent 30 cases of neonatal herpes in the United States annually at a cost of \$1.8 million per case prevented as well as 3.3 maternal deaths from complications of cesarean section deliveries.

Herpes simplex virus shedding from recurrent lesions and asymptomatic shedding can only be detected for 4 to 5 days.<sup>5-7</sup> Therefore unnecessary cesarean de-

liveries may be performed on the basis of a positive herpes simplex virus report of a previous week, and new episodes of asymptomatic shedding may occur subsequent to the last weekly test. Unfortunately, 70% of reported neonatal herpes simplex virus infections occurred in deliveries of mothers without apparent lesions or symptoms of genital herpes.<sup>8</sup>

Morgan and Smith<sup>9</sup> reported 65 of 83 herpes-positive cases were detected with a 4-hour, direct specimen, enzyme-linked immunosorbent assay by Ortho, with a sensitivity of 78.3%. Our conventional herpes culture method requires 24 hours to detect 62% or 48 hours to detect 87% of positive herpes simplex virus cultures. Detection of 100% of herpes simplex virus isolates still requires 7 days. We evaluated commercial herpes simplex virus antigen enzyme-linked immunosorbent assay kits during 1984 to determine whether these 4-hour methods could provide a reliable and more rapid laboratory diagnosis of herpes simplex virus than did cell culture.

## Material and methods

**Patient selection.** Obstetric patients with any of the following indications were tested for genital herpes simplex virus: (1) a prior history of genital herpes simplex virus, (2) a sexual partner with a history of genital herpes simplex virus, or (3) genital lesion(s) during pregnancy. These obstetric patients were tested at weeks 32, 34, and 36 and weekly thereafter until delivery. The sites cultured consisted of cervix (65.2%), vulva and lesions (21.2%), and cervix and vulva combined (13.6%). Only 17.9% of cultures indicated lesions present at the time of collection.

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**Table I.** Culture and enzyme-linked immunosorbent assay results, 1984

<i>Specimens</i>	<i>Enzyme-linked immunosorbent assay</i>	<i>Positive cultures</i>	<i>Negative cultures</i>	<i>Sensitivity (%)</i>	<i>Specificity (%)</i>
All patients	Positive	620	57	50.4	97.9
	Negative	609	2641		
Obstetric patients	Positive	108	56	34.3	98.1
	Negative	207	2866		

**Table II.** Enzyme-linked immunosorbent assay-positive and culture-negative results (optical density value percent above cutoff optical density\*)

	<i>+/-†</i>	<i>&lt;20%</i>	<i>&gt;20%</i>	<i>Total positive enzyme immunoassay only</i>
No.	14	10	32	56‡
%	25.1	17.8	57.1	100

\*  $\frac{\text{Optical density specimen}}{\text{Optical density cutoff}} \times 100 = \text{percent optical density value above cutoff.}$

† +/- = One optical density value above and one optical density below cutoff.

‡Eight (14%) patients had multiple enzyme-linked immunosorbent assay-positive and culture-negative specimens.

**Specimen collection and transport.** Specimens were collected on cotton and plastic swabs from the cervix, lesion(s), and/or vulva. Swabs were placed in human fibroblast Transporter tubes (Bartels Immunodiagnosics, Bellevue, Washington) as previously evaluated.<sup>10</sup> These specimens were transported in Styrofoam containers with ice packs. The transport time varied from 3 to 48 hours; 76% of all specimens were received <12 hours after collection, and only 1% of specimens were in transit for >36 hours.

**Cell culture methods.** Specimens in Transporters were agitated by Vortex mixer with glass beads after addition of 0.5 ml of antibiotic mixture consisting of 100 µg/ml of gentamicin sulfate, 250 µg/ml of amphotericin B, and 1000 µg/ml of vancomycin hydrochloride. An aliquot (0.3 ml) was inoculated into primary rabbit kidney cells, MRC-5 and/or human foreskin cells (Ortho Diagnostics, Carpinteria, California, and Bartels Immunodiagnosics). All inoculated culture tubes were examined once or twice daily for 7 days or until detection of cytopathic effect. All cultures developing cytopathic effect during the first 7 days were subpassaged and identified with use of either the "amplified" Ortho HSV antigen enzyme-linked immunosorbent assay on culture lysate and/or fluorescent antibody testing of infected cultures with herpes simplex virus types 1 and 2 monoclonal antisera (Syva, Palo Alto, California).

**Enzyme-linked immunosorbent assay methods.** Two commercial herpes simplex virus enzyme-linked immunosorbent assay antigen tests were evaluated dur-

ing 1984 (Ortho Diagnostics and Dako, Santa Barbara, California). Both kits used rabbit herpes simplex virus antisera conjugated to peroxidase enzyme. The chromogenic substrate used was o-phenylenediamine, and the intensity of the color reaction was measured with a Dynatech Minireader II spectrophotometer. All tests were performed according to the manufacturer's directions for the direct specimen test as previously described.<sup>11, 12</sup>

Obstetric specimens with sufficient volume were tested by both cell culture and the direct specimen enzyme immunoassay method with use of the Ortho herpes simplex virus antigen enzyme-linked immunosorbent assay system in duplicate. A portion of the specimens (209) were also tested with the Dako herpes simplex virus enzyme-linked immunosorbent assay.<sup>12</sup> Specimens with positive enzyme-linked immunosorbent assay and negative culture results were retested by both culture and enzyme immunoassay and also recollected when feasible. Only specimens which retested enzyme-linked immunosorbent assay positive and culture negative were considered false positive; specimens with insufficient volume for retest were excluded from the data.

The Ortho enzyme-linked immunosorbent assay positive cutoff value was adjusted in March according to manufacturer's directions as follows:

First positive cutoff value (1983 to 3/1984) = positive control mean - negative control mean  $\times 0.15$  + negative control mean

Second positive cutoff value (after 3/1984) = negative control mean - 0.10

The results of the Ortho enzyme-linked immunosorbent assay test were considered borderline if the two optical density values of the patient-specimen test well were above and below the cutoff value on the Ortho test. With the Dako test, results were considered borderline if test results did not meet both of the Dako positive criteria, that is, (1) the optical density of the test well minus the optical density of the negative control well is >0.1 and (2) the optical density of the test well divided by the optical density of the negative control well is >2.

**Neonatal herpes simplex virus infection.** Neonatal infection with herpes simplex virus was diagnosed in any baby who had herpes simplex virus isolated by culture from any body site during the first 28 days of life.

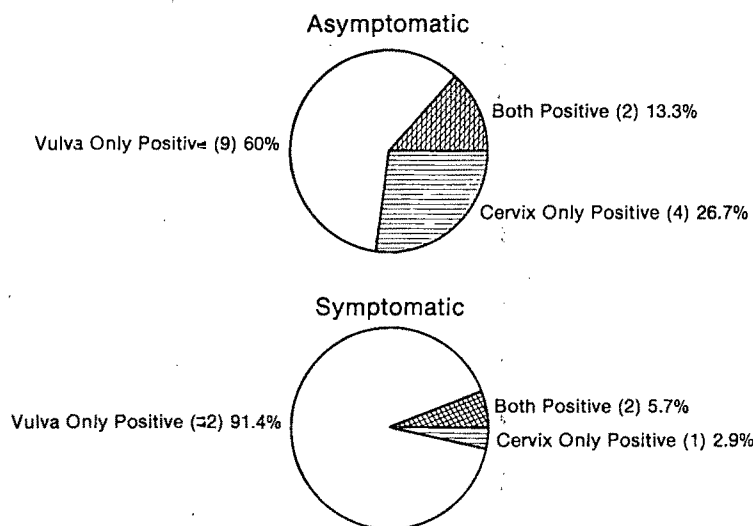


Fig. 1. Herpes simplex virus in paired specimens of cervix and vulva.

All charts were reviewed retrospectively. No cases were excluded. Virus cultures were performed when considered appropriate by the babies' attending physicians in a nonprospective fashion.

### Results

For an initial evaluation 3927 patient specimens obtained from men and from women, both pregnant and nonobstetric, were tested by cell culture and enzyme-linked immunosorbent assay for herpes simplex virus. These specimens were submitted from both outpatient gynecologic and urgent care clinics as well as from inpatients, primarily immunocompromised hosts or neonates. They consisted of genital swabs (74%) with urine, skin, and throat swabs totaling 15% of non-genital specimens. With these patient specimens the enzyme-linked immunosorbent assay sensitivity obtained was 50.4% and specificity was 97.9% (Table I).

A total of 3237 obstetric specimens were tested by enzyme-linked immunosorbent assay and cell culture, with a sensitivity of 34.3% and specificity of 98.1% (Table I). Sixty-four percent were from cervical swabs, and 82% were from asymptomatic obstetric patients.

Fifty-six obstetric patients had enzyme-linked immunosorbent assay-positive and culture-negative results from the same specimen. All of these false positive specimens were from cervical swabs. Of these, 32 of 56 were  $\geq 20\%$  above the positive cutoff optical density value, and eight patients (14%) had multiple enzyme-linked immunosorbent assay-positive and culture-negative results (Table II). Six of the enzyme-linked immunosorbent assay false positive results by the Ortho test were also tested by the Dako enzyme-linked immunosorbent assay, and only one of the six was positive by both tests.

A total of 23,719 women had live births during 1984, and 4773 (20%) of the births were by cesarean delivery.

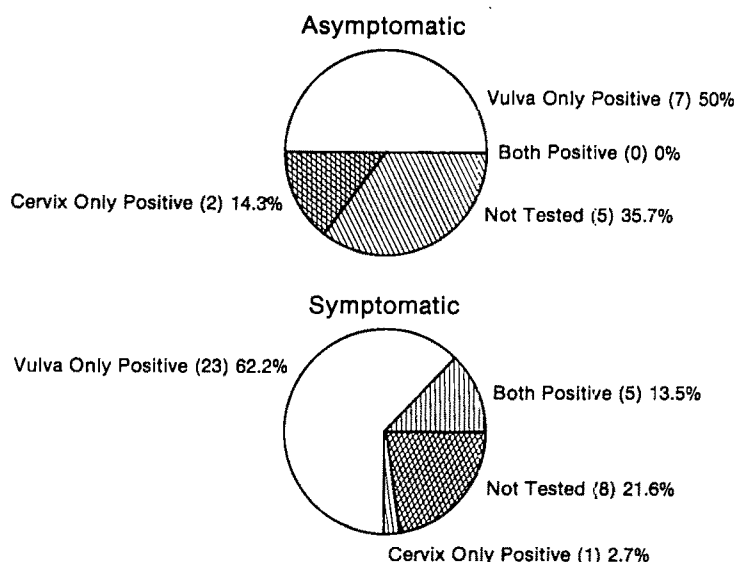
None of the infants of the 56 women with enzyme-linked immunosorbent assay-only positive results had any signs of herpes simplex virus disease or had herpes simplex virus recovered by cultures. Use of the enzyme-linked immunosorbent assay test alone without cell culture could have resulted in 1.7% (56 of 3237) unnecessary cesarean deliveries.

Paired cervical and vulvar specimens were collected from 195 obstetric patients, and herpes simplex virus was isolated from 50 (25.6%) in culture (Fig. 1). Vulvar specimens had a higher isolation rate of 41 of 50 (82%) as compared to cervical specimens, which had a 10% (five of 50) positive isolation rate. Herpes simplex virus was isolated more frequently from vulvar specimens of both symptomatic (32 of 35) and asymptomatic (nine of 15) patients. Herpes simplex virus was isolated from the cervix alone in 26.7% (4 of 15) of asymptomatic patients, compared to 2.9% (one of 35) of symptomatic patients.

When the enzyme-linked immunosorbent assay was used to test paired specimens, the vulvar specimens also had the highest positive rates—seven of nine in asymptomatic and 23 of 29 in symptomatic patients as compared to two of nine and one of 29, respectively, for cervical specimens (Fig. 2). From the paired specimens the overall sensitivity of vulvar specimens was 92.1% (35 of 38) as compared to 21.1% (eight of 38) with cervical specimens with use of the Ortho enzyme-linked immunosorbent assay test.

During 1984 six of the 23,719 babies delivered had herpes simplex virus isolated from culture specimens obtained during the first month of life, a rate of 25 per 100,000 live births. The source of herpes simplex virus in infected neonates was skin lesions in two, nasopharynx in two, urine in one, and multiple sites in one. In only the two babies with skin lesions was herpes simplex virus infection suspected at the time cultures were ob-





**Fig. 2.** Herpes simplex virus enzyme-linked immunosorbent assay of paired specimens of cervix and vulva.

tained. Clinical details of these six infants are summarized in Table III.

In Case 1 the infant had disseminated herpes simplex virus infection with fever, pneumonia, and disseminated intravascular coagulation. Cultures of blood, urine, and cerebrospinal fluid were all negative for bacteria. Surface cultures after the baby had expired yielded herpes simplex virus. Autopsy specimens and cultures confirmed herpes simplex virus infection involving brain, liver, and lungs. Three days after delivery this baby had developed a pustule on the occipital scalp at the fetal monitor site. Virus culture was not obtained from this lesion.

The infant in Case 2 was admitted to the hospital at the age of 9 days with vesicubullous lesions typical of bullous impetigo. Fluid from these lesions revealed gram-positive cocci in Gram stain and *Staphylococcus aureus* on bacterial culture. Viral culture yielded herpes simplex virus 5 days later, at which time the infant appeared healthy. All skin lesions had resolved during treatment with antistaphylococcal antibiotics.

In Cases 3 and 4 there was no evidence of cutaneous, ophthalmic, or disseminated herpes simplex virus at any time during observation. In Case 3, specimens for viral and bacterial culture were obtained within hours of delivery because of maternal fever. Surface cultures were not repeated before the start of antiviral therapy. In Case 4, cesarean-section was performed 2½ hours following spontaneous rupture of membranes, because of active genital herpes simplex virus infection. Unlike those in Case 3, these surface cultures were obtained from the baby 2 days after delivery.

In Case 5 the infant died at 10 weeks of age from

complications of bronchopulmonary dysplasia. At 3 weeks of age, a urine specimen for virus culture yielded herpes simplex virus. Although unexplained hepatomegaly was present from 10 days of age until death, significant elevations of transaminase levels were not noted until after administration of vidarabine. All other cultures from this patient, before and after vidarabine treatment, were negative for herpes simplex virus.

The infant in Case 6 developed typical herpetic vesicles at 11 days of age while still in the hospital for management of hydronephrosis from posterior ureteral valves. The mother had experienced the first symptoms of genital herpes during week 30 of pregnancy. Cervical cultures for herpes simplex virus, both 3 and 17 days before delivery, were negative. No herpetic lesions were present at the time of delivery. A cesarean section was performed for failure to progress at 16 hours after spontaneous rupture of membranes. Treatment with acyclovir was administered to the baby from day 11 through day 21, with rapid resolution of vesicles. However, similar lesions, again yielding herpes simplex virus on culture, reappeared on day 28. Acyclovir was again administered from day 28 through day 38. No further recurrences were noted.

Of these six neonates infected with herpes simplex virus, only two mothers had been tested prenatally for herpes simplex virus. Both had negative cervical cultures, preceded by positive vulvar cultures. The mothers not tested lacked either a history of herpes simplex virus risk factors or prenatal care.

In addition, a seventh baby (Case 7) was evaluated because of a positive herpes simplex virus culture obtained from amniotic fluid at delivery. The previous

birth for the 33-year-old mother, para ., gravida 2, abortuses 0, had been by cesarean section because of genital herpes. During the second pregnancy, she had no symptoms of herpes simplex virus. A cervical culture done 25 days before delivery was negative for herpes simplex virus. No genital lesions were present at delivery. This baby was delivered by elective repeat cesarean section. Culture specimens from the baby's eye, pharynx, blood, cerebrospinal fluid, and urine performed on day 10 were all negative for herpes simplex virus. At no time did the baby exhibit clinical or laboratory evidence of herpes simplex virus infection. Anticipatory vidarabine therapy was administered from day 10 through day 17 of life, while awaiting culture results.

### Comment

The herpes simplex virus antigen enzyme-linked immunosorbent assay method was compared to conventional cell culture to determine whether it could provide comparable results in hours instead of days. As in other centers,<sup>2</sup> the number of neonatal herpes simplex virus infections in our population has recently increased, from no cases in 1980 to two cases in 1982 and in 1983 and again to six cases in 1984, with a stable number of live births. A rapid test for herpes simplex virus, if sensitive and specific, would permit accurate identification of infectious mothers. The result would be the limitation of cesarean deliveries to cases in which one is actually necessary for prevention of neonatal disease. Such a test would also be useful in the evaluation of women who have had no routine prenatal care.

In our initial evaluation of the commercial herpes simplex virus antigen enzyme-linked immunosorbent assay the overall sensitivity was found to be 50.4% with use of both male and female genital specimens. The enzyme-linked immunosorbent assay sensitivity varied with regard to specimen source, ranging from 100% for buttock lesions to 21.7% for asymptomatic cervical specimens as previously reported.<sup>10</sup> The sensitivity of the enzyme-linked immunosorbent assay also varied directly with the amount of virus present in the specimen; 71.4% of cultures which developed cytopathic effect on day 1 were detected by enzyme-linked immunosorbent assay whereas only 13.3% of cultures with cytopathic effect detected on days 4 through 7 after inoculation were positive by the assay.

For obstetric patients the sensitivity of the enzyme-linked immunosorbent assay was only 34.3%. This lower sensitivity reflects the predominance of asymptomatic cervical specimens. Only 21.8% of nonobstetric female genital specimens are from cervical swabs as compared to 65.2% of obstetric specimens. Also non-obstetric cervical specimens had a 28.6% herpes simplex virus isolation rate as compared to only 5.3% positive specimens from obstetric cervical swabs.

The 56 enzyme-linked immunosorbent assay false positive results were obtained from obstetric cervical specimens. These enzyme-linked immunosorbent assay—only positive results may be due to one or more of the following: (1) technical error—conjugate carryover because of poor washing technique, (2) nonspecific reactivity of the polyclonal rabbit antibody, and (3) non-infectious herpes simplex virus with infectivity lost in transit or noninfectious herpes simplex virus antigen from the host.

The enzyme-linked immunosorbent assay method requires a consistent washing technique to obtain reproducible results. Even with use of duplicate specimen wells, which doubled the cost of the assay, borderline results were still found to be nonreproducible in 60% of repeated tests. The specificity of the enzyme-linked immunosorbent assay test improved with experience; the specificity for the first 3 months was 96.9%, and for the last 9 months was 98.4%. This maximum specificity was attained only by the use of duplicate results, with discarding of nonreproducible positives after repeat tests. The use of additional positive and negative controls daily did not indicate presence or absence of sporadic nonreproducible false positive results.

Since the capture antibodies provided in these enzyme-linked immunosorbent assay kits are polyclonal rabbit immunoglobulins, the possibility of nonspecific reactions exists. Yolken<sup>13</sup> reports that nonspecific reactivity is a problem particularly with the herpesvirus group and mammalian cell membranes. Although cervical specimens represented 63.5% of specimens tested, all false positive specimens in obstetric patients were from cervical swabs.

Only infectious virus is isolated in cell culture, but the enzyme-linked immunosorbent assay detects non-infectious herpes simplex virus antigen material. Some of the false positive specimens may be noninfectious herpes simplex virus. However, all obstetric specimens were inoculated directly into fibroblast cells in the Transporter tubes at bedside. Also, none of the false positive specimens were in transit for more than 18 hours. It is more likely that the noninfectious antigen material was collected from the patients.

Thirty-two false positive specimens had high enzyme-linked immunosorbent assay optical density values (more than 20% above cutoff), and eight women had multiple enzyme-linked immunosorbent assay—only positive specimens; although nonspecific reactions cannot be excluded, noninfectious herpes simplex virus antigen is more likely to be found in these women with a history of genital herpes.

The herpes culture results were used to determine management of the obstetric patients, and none of the babies of mothers whose enzyme-linked immunosorbent assay results were false positive developed clinical



**Table III.** Herpes simplex virus infection in six neonates

Case No.	Maternal data						
	Age (yr)	Parity	Gestational age	Type of delivery	Prenatal care	At risk for herpes simplex virus*	Time from rupture of membranes to delivery (hr)
1	14	G1P0	Term	Vaginal	No	No	21
2	23	G4P2Ab1	Term	Vaginal	Yes	No	1½
3	13	G1P0	34 wk	Vaginal	Yes	No (raped)	7
4	22	G3P1Ab1	Term	Abdominal (active lesions)	Yes	Yes	2½
5	35	G2P1Ab0	Term	Abdominal (failure to progress)	Yes	Yes	16
6	23	G2P0Ab1	32 wk	Vaginal	Yes	No	48

G = Gravida; P = para; Ab = abortuses; NP = nasopharynx swab; CSF = cerebrospinal fluid.

\*Positive history for genital herpes simplex virus in patient or sexual partner or for genital lesions during pregnancy.

†Postmortem cultures.

signs of herpes simplex virus disease or any positive herpes simplex virus cultures after birth. If the enzyme-linked immunosorbent assay results alone were used to determine management, the increase in cesarean deliveries could have been 1.7%.

With use of paired vulvar and cervical swabs from symptomatic and asymptomatic obstetric patients, herpes simplex virus shedding was detected eight times more often from vulvar than from cervical specimens. With asymptomatic patients, cervical swab specimens were positive in only 40% of cases, whereas vulvar swab specimens were positive in 73%. Previous investigations<sup>6,7</sup> have reported asymptomatic herpes simplex virus shedding detected more often from vulvar swabs, and these investigators have suggested that both sites be cultured in pregnant women. Since two cases of neonatal herpes occurred in women with negative cervical cultures the week of delivery preceded by positive

vulvar culture, we also recommend the collection of both cervical and vulvar swabs weekly.

The use of vulvar specimens was found to improve both the sensitivity and specificity of the enzyme-linked immunosorbent assay test. However, the maximum sensitivity obtained with vulvar specimens from obstetric patients was 71.7%. False positive results were never eliminated, with three cases occurring after 12 months of enzyme-linked immunosorbent assay experience and with the use of duplicate specimen test wells.

The incidence of neonatal herpes simplex virus infection in this population, 25 per 100,000 live births, is comparable to that reported elsewhere: 26 per 100,000 from 1976 through 1980<sup>2</sup> and 28 per 100,000 in 1982.<sup>14</sup> However, our cases reflect a much smaller incidence of disseminated infection. This may be due in part to the early institution of antiviral therapy. Infants in Cases 3, 4, and 6 received therapy for positive

Neonatal data					
Birth date	Herpes simplex virus-positive site	Date of cultures	Herpes simplex virus-negative site	Date of cultures	Antiviral therapy
2/10	Eye NP Brain Liver Lung Vesicle	2/18 2/18 2/22† 2/22 2/22 2/12	Rectal	2/18	None
2/3			Eye NP Rectal Blood CSF CSF Skin	2/16 2/16 2/16 2/17 2/17 2/27 2/28	Acyclovir, 10 days
6/4	NP	6/4	CSF CSF Blood Blood Rectal	6/4 6/14 6/7 6/8 6/4	Acyclovir, 10 days
12/6	NP	12/3	NP CSF Urine Eye Eye	12/17 12/17 12/17 12/8 12/17	Vidarabine, 10 days
7/13	Vesicle Vesicle	7/24 8/10	CSF Blood Rectal NP Eye Urine Skin	7/24 7/24 8/10 7/24 7/24 7/24 7/30	Acyclovir, two courses of 10 days each
9/13	Urine	10/6	Urine Urine CSF NP Rectal	9/19 10/12 10/12 10/12 10/13	Vidarabine, 10 days

herpes simplex virus cultures, two from nasopharynx and one from urine, without manifesting any evidence of cutaneous or systemic herpes simplex virus disease. In Case 5 therapy was initiated as soon as cutaneous lesions appeared. Since vesicles, and presumably positive cultures, can precede dissemination by several days,<sup>8</sup> it is possible that systemic disease was prevented in some of these infants. In addition, in Case 3 the isolation of herpes simplex virus within hours of delivery could reflect transient contamination rather than infection. Case 7, with demonstration of herpes simplex virus in amniotic fluid but with no evidence of herpes simplex virus colonization or disease by 10 days of age, further illustrates the point that exposure of an infant to herpes simplex virus does not necessarily lead to demonstrable infection. Therefore it is currently recommended that cultures of mucous membranes be obtained 24 to 36 hours after birth to differentiate herpes simplex virus infection from transient contamination of the neonate.<sup>15</sup>

These cases also reveal the diversity of clinical problems that herpes simplex virus can cause in neonates. Whether or not the scalp lesion in Case 1 was an atypical herpetic vesicle, disseminated herpes simplex virus infection can present as sepsis with negative bacterial and fungal cultures.<sup>16</sup> Vesicles may not be present at any time during the illness.<sup>8</sup> When vesicles are present, they can mimic vesiculobullous eruptions of other causes.<sup>16</sup> With the apparent increase in incidence of genital and neonatal herpes simplex virus infection, it is important to remember the atypical presentations as well as the common.

In conclusion, vulvar specimens were found to be superior to cervical ones for the detection of asymptomatic shedding of herpes simplex virus in both culture and enzyme-linked immunosorbent assay. The herpes simplex virus enzyme-linked immunosorbent assay antigen methods studied were not as sensitive as conventional cell culture, particularly in asymptomatic patients. The use of the direct specimen herpes simplex



virus antigen enzyme-linked immunosorbent assay alone would have increased the cesarean delivery rate but would not have reduced the incidence of neonatal herpes simplex virus.

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# The perinatal and economic impact of prenatal care in a low-socioeconomic population

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Reductions in publicly funded prenatal care programs in 1981 to 1984 resulted in an increase in unregistered patient deliveries from 7.8% to 14.9% of births at University of California San Diego Medical Center. To assess the economic and perinatal impact of the increasing number of deliveries of women without prenatal care, 100 consecutive patients with fewer than three prenatal visits were studied. Each "no care" patient was matched by age, parity, and week of delivery with a control patient who received care in a state-funded perinatal project (Comprehensive Perinatal Program). Maternal antenatal risk factors were equally distributed between the two groups when maternal age, parity, history of substance abuse, prior preterm delivery, hypertension, and abortion were compared. Maternal obstetric outcomes were similar, including cesarean section rate and incidence of postpartum fever and hemorrhage. However, neonates delivered of women receiving no care experienced significantly greater morbidity than the neonates of women in the Comprehensive Perinatal Program, including an increased incidence of premature rupture of the membranes and preterm delivery (13% versus 2%,  $p < 0.05$ ), low birth weight (21% versus 6%  $< 2500$  gm,  $p < 0.002$ ), and intensive care unit admissions (24% versus 10%,  $p < 0.005$ ). When the total inpatient hospital charges were tabulated for each mother-baby pair, the cost of perinatal care for the group receiving no care (\$5168 per pair) was significantly higher than the cost for patients in the Comprehensive Perinatal Program (\$2974 per pair,  $p < 0.001$ ) including an antenatal charge of \$600 in the Comprehensive Perinatal Program. The excess cost for delivery of 400 women receiving no care per year in the study hospital was \$877,600. These results suggest that extension of prenatal care programs to medically indigent women is likely to result in a net reduction in perinatal morbidity and health care expenditures. (AM J OBSTET GYNECOL 1986;154:29-33.)

**Key words:** Prenatal care, prematurity, midwifery

The association between lack of prenatal care and increased maternal and fetal morbidity has been recognized for almost half a century. Eastman<sup>1</sup> observed, in 1947, that the prematurity rate was 24% among patients at The Johns Hopkins Hospital who received no prenatal care but only 8% among women with three or more prenatal visits. This finding was confirmed by several subsequent investigators<sup>2-7</sup> but disputed by others.<sup>8-10</sup> Perhaps because of the controversy regarding the cost-effectiveness of antenatal care, the concept of "guaranteed access" to prenatal services has not been universally embraced.<sup>11</sup> Moreover, participation in antenatal programs varies widely among subpopulations.<sup>12, 13</sup> In California, from 1978 to 1982, 10.4% of all deliveries were of women who received no prenatal care, unknown care, or third-trimester care only.<sup>14</sup>

At the University of California San Diego Medical Center, "no care" deliveries present a special problem. Situated near the United States-Mexico border, the hospital acts as a primary care center for a large number of women of Mexican descent. A small fraction of patients receive care from private physicians or Health Maintenance Organizations (12%), and a somewhat larger proportion of patients qualifying for state-funded medical care (Medi-Cal) receive prenatal care in the UCSD Clinics (22%). However, the remaining patients are indigent and ineligible for the usual sources of publicly funded medical care, principally because of their status as undocumented aliens (66% of all patients delivered). The only low-cost prenatal service available to these patients is the Comprehensive Perinatal Program, a state-funded, nurse-midwife-staffed program that operates within a consortium of 10 community clinics throughout San Diego County.

However, enrollment in the Comprehensive Perinatal Program is limited to 100 patients per month. Because the potential Comprehensive Perinatal Program subscribership is more than 200 patients per month, an extensive waiting list for enrollment has resulted. Patients unable to enroll in the Comprehensive

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**Table I.** Maternal antenatal data

Factor	No care (Mean $\pm$ SD) (%)	Care (Mean $\pm$ SD) (%)
Total Deliveries	100	100
Mean age	23.8 $\pm$ 2.3	24.8 $\pm$ 5.0
<18 yr	10	5
>35 yr	3	3
Nulliparity	39	33
Obstetric history		
Hypertension	2	2
Substance abuse	10	3
Prior preterm delivery	2	4
Pregnancy loss or abortion	24	30
Foreign citizenship	75	96
Eligible for Medi-Cal	28	26
Pregnancy duration		
Mean (wk)	39.4 $\pm$ 4.0	40.1 $\pm$ 1.0*
Preterm (%)	13	2*
Prenatal visits		
Mean No.	1 $\pm$ 1	12 $\pm$ 3*
First visit (wk)	39.5 $\pm$ 1.2	14.0 $\pm$ 3.6*

\* $p < 0.01$ .

Perinatal Program continue their pregnancies without prenatal care and frequently present to UCSD Medical Center for delivery. During the 3 years preceding the present study, the number of "no care" deliveries at UCSD increased almost fourfold. This study was undertaken to assess the perinatal and economic impact of the rising numbers of "no care" deliveries by comparing in detail the outcomes of women who received prenatal care in the Comprehensive Perinatal Program with outcomes in those who did not ("no care").

### Methods

**Patient selection.** One hundred consecutive deliveries at UCSD Medical Center of women who received no prenatal care during pregnancy were identified. Patients who received prenatal care at another institution or who were seen in an emergency room on more than two occasions during pregnancy were excluded. For comparison, each "no care" patient was matched by week of delivery with a patient who received prenatal care in the Comprehensive Perinatal Program. To ensure that the Comprehensive Perinatal Program group received adequate prenatal care, patients whose first prenatal visit occurred after 20 weeks were excluded from the study.

**Prenatal care.** Prenatal care in the Comprehensive Perinatal Program was provided by certified nurse-midwives in a network of 10 outlying community clinics. The Comprehensive Perinatal Program clinics provide a comprehensive program of perinatal care and education that includes obstetric care, nutritional assessment and support, social service, and home-outreach services. With the exception of insulin-requiring diabetics, no patients were transferred from the Compre-

hensive Perinatal Program because of antenatal medical or obstetric complications. Patients with complications were presented by the midwife to staff physicians regularly available for consultation at the clinic site. Additionally, the case records of patients with complications were reviewed weekly at a high-risk conference by a panel consisting of a perinatologist, the nurse-midwife staff, program dietitians, social workers, and clinic managers. Antepartum testing and sonography services were provided to Comprehensive Perinatal Program patients at no additional cost. Patients were asked to reimburse the clinic a single "packaged" fee (\$300 to \$675, on a sliding scale depending on ability to pay) for prenatal and delivery-postpartum care.

**Intrapartum management.** After admission to the delivery unit, "no care" patients were managed by the obstetric house staff and faculty. Comprehensive Perinatal Program patients were usually managed and delivered by certified nurse-midwives from the Comprehensive Perinatal Program. Labors complicated by maternal medical conditions, fetal distress, prematurity, or dystocia were managed in conjunction with the obstetric staff. However, Comprehensive Perinatal Program patients managed or delivered by obstetric staff were considered to be in the Comprehensive Perinatal Program group for the purposes of the study.

**Data collection and analysis.** The maternal and newborn medical records of the 100 Comprehensive Perinatal Program and "no care" pairs were reviewed. Antenatal, postpartum, and newborn outcome data were abstracted and coded for analysis. In cases in which the duration of gestation at delivery was unclear, the Dubowitz evaluation of the newborn infant was used to corroborate the obstetric history. The total charges billed to each mother and baby during the course of their hospital stay were obtained from the hospital's billing unit. The  $\chi^2$  and Student's  $t$  tests were used to evaluate the significance of differences between the two groups.

### Results

Selected antenatal maternal risk factors for the "no care" and Comprehensive Perinatal Program patients are listed in Table I. Mean maternal age was similar in both groups, as was the relative proportion of women under the age of 18 or over 35. Nulliparous patients were relatively evenly distributed between the study populations, as were patients with histories of previous preterm delivery, pregnancy loss, elective abortion, hypertension, and substance abuse. The remaining demographic variables (United States citizenship and eligibility for publicly funded medical care) were similar as well.

Table I also compares the number of prenatal visits to a clinic or emergency room as well as the mean week

**Table II.** Neonatal complications

Complication	No care (Mean $\pm$ SD) (%)	Care (Mean $\pm$ SD) (%)	p Value
Premature rupture of membranes	13	2	0.006
Meconium	19	25	NS
Ominous fetal heart rate tracing	10	5	NS
Prematurity (<37 wk)	13	2	0.006
Birth weight			
Mean (gm)	3087 $\pm$ 256	3385 $\pm$ 476	0.01
<2500 gm (No.)	21	5	0.004
5 min Apgar score <7	8	2	0.07
Hospital stay >3 days	24	12	0.05
Perinatal death	4	1	0.11

of pregnancy in which the first visit occurred. The Comprehensive Perinatal Program patients had a mean of 12 prenatal visits (versus  $1 \pm 1$  for the "no care" population) beginning in the fourteenth gestational week (versus the thirty-ninth week among the "no care" patients). This was the only antepartum risk factor that was significantly different between the two groups ( $p < 0.01$ ).

Intrapartum and postpartum management of patients was similar in that the frequency of oxytocin, epidural anesthesia, and antibiotic usage intrapartum was comparable. A trend to higher cesarean delivery rates was evident in the Comprehensive Perinatal Program group (14% versus 9%), but the incidence of instrument-assisted delivery was almost identical (22% versus 20%, Comprehensive Perinatal Program and "no care" groups, respectively,  $p = \text{NS}$ ). Maternal postpartum morbidity as a whole (18% in both groups) and when analyzed by individual complications was relatively similar. An exception was postpartum transfusion for severe anemia (hematocrit <28% in seven "no care" women versus one Comprehensive Perinatal Program patient;  $p < 0.05$ ). The mean hospital stay of "no care" patients was approximately 1 day longer than that of the Comprehensive Perinatal Program group (3.6 versus 2.4 days,  $p < 0.01$ ).

In contrast to the comparable maternal outcomes, the fetal and newborn complications (Table II) were distinctly dissimilar. The differences in the incidence of meconium-stained amniotic fluid and ominous fetal heart rate tracings were not significant, but "no care" patients were much more likely than Comprehensive Perinatal Program patients to be admitted with premature rupture of the membranes and premature labor (13% versus 2%,  $p < 0.01$ ). The higher frequency of preterm delivery among the "no care" patients is reflected in the increased incidence of low birth weight (<2500 gm in 21% of "no care" patients and 6% of Comprehensive Perinatal Program patients;  $p < 0.005$ ) and prolonged newborn hospital stay (>3 days in 24%

**Table III.** Hospital charges

	No care (Mean $\pm$ SD) (%)	Care (Mean $\pm$ SD) (%)
Maternal charges *	\$1722 $\pm$ 35	\$1663 $\pm$ 38
Neonatal charges *	\$3487 $\pm$ 599	\$ 697 $\pm$ 48
Total charges	\$5168	\$2374

\* $p < 0.001$ .

**Table IV.** Costs and projected savings

Excess cost per no care delivery	\$2794
Cost of Comprehensive Perinatal Program professional services	600
Projected savings with prenatal care (per patient delivered)	\$2194
Projected savings with prenatal care (400 patients per year)	\$877,600

of "no care" patients and 12% of Comprehensive Perinatal Program patients;  $p < 0.05$ ). Infants of "no care" women also experienced a somewhat increased risk of a low 5-minute Apgar score (<7 in 8% of "no care" patients and 2% of Comprehensive Perinatal Program patients;  $p = 0.07$ ).

Four perinatal deaths, all occurring before admission, were detected in the "no care" group. Three patients presented with uncontrolled hypertension, vaginal bleeding, and fetal death. Abruptio placentae was diagnosed in these patients at delivery. The fourth patient was admitted with chorioamnionitis and fetal death. In the Comprehensive Perinatal Program group, a single intrapartum death occurred. This patient was instructed to ambulate in early labor after auscultation of normal fetal heart tones. One hour later, thick meconium staining and fetal death were documented. The autopsy results were inconclusive. The difference in fetal death rates between these groups was not statistically significant.

The hospital charges generated by the care of each mother and baby were tabulated for the period from admission in labor until discharge (Table III). The difference in maternal hospital charges between the "no care" group and Comprehensive Perinatal Program patients was small (mean difference = \$59;  $p < 0.001$ ). On the other hand, mean neonatal charges were strikingly higher in the "no care" group (\$3487 in "no care" group versus \$697 in Comprehensive Perinatal Program patients;  $p < 0.001$ ). When the mean of total charges accrued in providing intrapartum and postpartum care to each mother-baby pair was assessed, the typical "no care" patient bill was \$5168 compared with \$2347 for a Comprehensive Perinatal Program delivery. The average cost per delivery was \$2821 ( $p < 0.001$ ).



**Table V.** Preterm deliveries: No care and Comprehensive Perinatal Program patients

Patients	Year 1 (N = 757)		Year 2 (N = 1048)		Year 3* (N = 1410)	
	n	%	n	%	n	%
Comprehensive Perinatal Program	16/534	3.0	29/711	4.1	49/1034	4.7
No care	32/223	14.3†	35/337	10.4†	38/376	10.1†

\*Study year.

† $p < 0.001$ .

**Cost analysis.** During the study period, the cost of providing comprehensive perinatal care in the Comprehensive Perinatal Program was \$600 per patient. This cost included antenatal professional fees (routine visits, performance and interpretation of nonstress tests and sonography) and intrapartum and postpartum professional services provided by the Comprehensive Perinatal Program midwives and/or obstetric staff. Adding the \$600 care cost to the overall charges for a Comprehensive Perinatal Program delivery results in a total Comprehensive Perinatal Program obstetric expense of \$3000 per case. When this expense is compared with the \$5168 cost for a "no care" delivery, the net excess expenditure per "no care" patient was approximately \$2100. At the rate of 400 "no care" deliveries per year in the study institution, the annual excess cost of delivering these patients was \$877,600 (Table IV).

### Comment

This study assessed the perinatal and economic impact of prenatal care on maternal and neonatal outcome in a cohort of indigent women. In this population, provision of prenatal care did not appear to reduce appreciably the incidence of maternal complications or morbidity associated with labor and delivery. However, infants delivered of "no care" women experienced significantly greater perinatal morbidity and mortality, primarily associated with increased prematurity. The preterm (<37 weeks) delivery rate was 13% among the "no care" patients versus 2% in the Comprehensive Perinatal Program. The hospital charges resulting from the care of these infants exceeded by severalfold the cost of providing prenatal care to their mothers.

The preterm delivery rate of 13% in the "no care" group is consistent with the results reported in other studies. Tokuhata et al.<sup>3</sup> studied birth certificate data of 185,000 deliveries and found a 23.6% prematurity rate among women without prenatal care compared with 6.9% among those with care. Similar results were obtained by Bruce et al.<sup>4</sup> (22.9% versus 9.5% prematurity), as well as Klein<sup>5</sup> (33.1% versus 9.1%), Ryan et al.<sup>6</sup> (15.8% versus 9.9%), and Greenberg<sup>7</sup> (2.5 times increased risk of prematurity). Kaunitz et al.<sup>15</sup> recently

reported a threefold increase in perinatal mortality among women who, for religious reasons, avoided prenatal care and practiced home birth when compared with women managed by physicians in a hospital setting. However, these studies were not well controlled for obstetric risk factors such as race, substance abuse, previously poor obstetric outcome, and social stratum. Moreover, patients in the "no care" groups were often sociologically and demographically distinct from the groups receiving prenatal care. Indeed, the "no care" women often refused or avoided prenatal care because of drug addiction, alcoholism, or transitory lifestyle.<sup>3-5</sup> In the present study, however, the reason for lack of care was, in general, lack of availability of antenatal services. Thus the populations compared in this study appear to be drawn from a relatively homogeneous pool of medically indigent women.

The finding of only 2% prematurity in the Comprehensive Perinatal Program group (compared to the 6% to 8% rate observed in most centers) was striking. The unusually low rate in the Comprehensive Perinatal Program could be due to (1) a population with an intrinsically low risk of preterm delivery, (2) a bias in ascertainment of the Comprehensive Perinatal Program population by exclusion of patients who registered for prenatal care after 20 weeks, or (3) a type I statistical error.

To clarify these issues, the preterm delivery rate in the entire Comprehensive Perinatal Program (including patients initiating care at all gestational ages) was evaluated during the year in which the study was conducted as well as the two preceding years. The preterm delivery rate was also assessed in the "no care" population. This analysis (Table V) demonstrated that the preterm delivery rate for "no care" patients was significantly higher than that of the Comprehensive Perinatal Program patients in all 3 years studied (mean rate of 3% in Comprehensive Perinatal Program group versus 12% in "no care" patients;  $p < 0.001$ ). Further, the Comprehensive Perinatal Program patients regularly achieved a low prematurity rate even when late-registering patients were included (approximately 65% of Comprehensive Perinatal Program patients register after 20 weeks' gestation). It is therefore unlikely that the

outcome of the cohort of patients examined in detail in the study protocol is unrepresentative of the population as a whole. When the strength of the difference between preterm delivery rates of the two groups ( $p = 0.006$ ) is considered, it is also not likely that a type I error could have occurred.

There were no demonstrable differences in obstetric and demographic risk factors between the "no care" and the Comprehensive Perinatal Program group. Detailed comparisons indicate that the Comprehensive Perinatal Program and "no care" patients were indigent, predominantly Hispanic women with relatively limited access to prenatal care. Indeed, at the time of delivery, many of the "no care" patients were on a waiting list to enter the Comprehensive Perinatal Program. The limitation in Comprehensive Perinatal Program enrollment to 100 patients per month and ineligibility of the remaining patients for other publicly funded prenatal care programs resulted in an "overflow" of approximately 100 women per month into the "no care" pool. These patients continued to request and receive delivery and postpartum services despite their lack of prenatal care.

A final question may be raised as to whether limiting the study group to patients who initiated care before 20 weeks exaggerated the differences in gestational length and neonatal morbidity between the two groups. This approach was taken deliberately in order to assess the specific effect of comprehensive prenatal care on outcome. Thus it was considered essential to ensure the Comprehensive Perinatal Program group received full prenatal care and to exclude patients who may have been seen only for a few visits.

A new finding in this study was the markedly higher financial cost of delivering patients without prenatal care when compared with that of the patients given care. Detailed analysis of patient charts and hospital billing records in this study demonstrated that the cost of care of premature "no care" newborn infants far exceeds the cost of comprehensive maternal antenatal care. Even if the cost of professional services in the Comprehensive Perinatal Program (\$600) is relatively low, the cost differential of \$2168 per "no care" delivery would provide significant net savings even if more costly physician care (estimated cost \$750 to \$1200) were extended to the "no care" patients.

The implications of these results for economic and health policies are numerous. In the population studied

here, expansion of prenatal care programs to include presently ineligible women would likely engender significant savings in delivery and nursery costs as well as result in decreased infant morbidity and mortality. Because labor, delivery, and newborn care is ultimately provided to these indigent women regardless of their antenatal care status, investment in prenatal surveillance would seem to be a more cost-effective approach. Furthermore, the expected decrease in newborn care expenditures does not consider the enormous potential savings in aftercare (special education, follow-on medical support) frequently required in the first years of a premature infant's life or the "hidden costs" of preterm birth, that is, the considerable psychological stresses on the families involved.

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# A randomized study of three cannulas for transcervical chorionic villus sampling

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A randomized trial involving 200 transcervical chorionic villus samples taken with three different cannulas was undertaken. In terms of karyotype recovery and ease of insertion the aluminum cannula performed best although the placental site influenced the ability to recover villi for all cannulas. (AM J OBSTET GYNECOL 1986;154:34-9.)

**Key words:** Chorionic villus sampling, cannulas, placental site

If chorionic villus sampling eventually replaces amniocentesis as the preferred technique for the prenatal diagnosis of genetically inherited disorders, then it is likely that a transcervical approach will be used. In a recent survey, 55 of 56 centers performing diagnostic chorionic villus sampling used a transcervical route.<sup>1</sup> The most favored method was that first described by Ward et al.<sup>2</sup> With this method a plastic cannula with an inner aluminum obturator (Portex Tropochan) is passed through the cervix into the uterus and directed to the placental site under continuous real-time ultrasound control. The aluminum obturator, which adds malleability to the device and enhances its appearance on the ultrasound image, is withdrawn and suction is applied and maintained as the plastic cannula is withdrawn.

There are problems with this method and with the design of the cannula. The main problem is the stiffness of the plastic cannula with a loss of rigidity and shape after the aluminum obturator is withdrawn. Similarly, accurate visualization of the tip of the cannula is not possible with ultrasound guidance and definition is lessened after the aluminum obturator is removed. In an attempt to remedy these problems we have designed two cannulas. In one, made of aluminum throughout, the tip is rounded and has two opposing "eyes" through which villi can be sucked. In the other, made of stainless steel, there is an olive-shaped tip, which was designed to enhance its visualization on the ultrasound image.

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**Table I.** Characteristics of cannulas

Cannulas	Length (mm)	Internal diameter (mm)	Outer diameter (mm)
Portex Tropochan	210	1.13	1.45
Malleable stainless steel	210	1.80*	2.70
Aluminum	210	1.30	2.30

\*At olive tip.

This tip also contains two "eyes" through which villi can be sucked.

We have compared these cannulas and the Portex Tropochan in a random study with special reference to villus recovery rates and subsequent karyotyping, as it is in the field of chromosomal diagnosis that chorionic villus sampling is likely to find its widest application.

## Material and methods

**Cannulas.** Three cannulas were used. The Portex Tropochan, a malleable stainless steel cannula, and an aluminum cannula, Birmingham Hospital Pattern, both made by Rocket of London.

Details of the cannulas are given in Table I and their design is seen in Fig. 1.

**Patients.** Fifty patients between 8 and 12 weeks' gestation, as assessed on ultrasound measurement (mean  $9.4 \pm 0.7$  weeks), were enrolled into the study. All were undergoing termination of pregnancy for nongenetic reasons. Informed consent was obtained in every case. Local ethical committee approval was obtained. Only viable pregnancies as demonstrated by ultrasound were studied. One twin pregnancy was excluded from the study. Sampling was performed in the 24 hours prior to termination.

**Sampling method.** No anesthesia or analgesia was used before or during sampling. Prior experience had been gained by one of us (W. E. MacKenzie) in obtaining chorionic villi by the transcervical technique de-

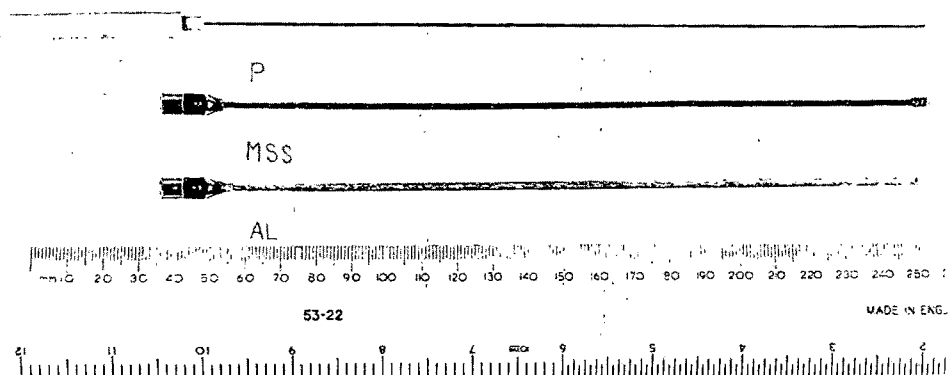


Fig. 1. Photograph of three types of cannulas. P, Portex Trophocan; MSS, Malleable stainless steel cannula; AL, aluminum cannula.

scribed by Ward et al.<sup>2</sup> This involved obtaining 128 samples from 71 patients.

In the present study the chorionic villus sampling was carried out by the operator (W. E. MacKenzie) who used the ultrasound transducer to guide the cannula to the sampling site. Preliminary ultrasonography (with the ADR 4000 sector scanner and a 3 MHz probe) was carried out to verify gestational age of the fetus by crown-rump length measurements and to confirm viability. The placental site was visualized and assigned to a predominantly anterior or posterior uterine position.

A sterile Cusco speculum was passed into the vagina and the cervix was visualized. The position and angle of the uterocervical canal were assessed and the cannula bent to the same angle. With the use of continuous real-time ultrasound guidance, the cannula was passed through the cervix and the image of the cannula tip observed. Under ultrasound guidance the cannula tip was maneuvered to the placental site, which was entered to a depth of approximately 1 cm. to overcome the problem of sampling degenerating villi at the placental edge. Then 10 cm<sup>3</sup> of suction was applied and with a slight vacuuming action and with suction maintained the cannula was withdrawn. Each sample was collected into 5 ml of F10 medium containing 20% fetal calf serum, for subsequent cytogenetic analysis. Each cannula was passed twice and this was designated a "procedure." Ward (Ward RHT. Paper presented at the first international symposium on chorionic villus sampling, Nottingham, England, 1984) had shown that two passages of the Portex cannula were associated with an 80% villus recovery rate and were considered sufficient for this study.

**Random allocation of cannulas.** Prior to the commencement of the study one of the three types of cannula was selected at random and assigned to a proce-

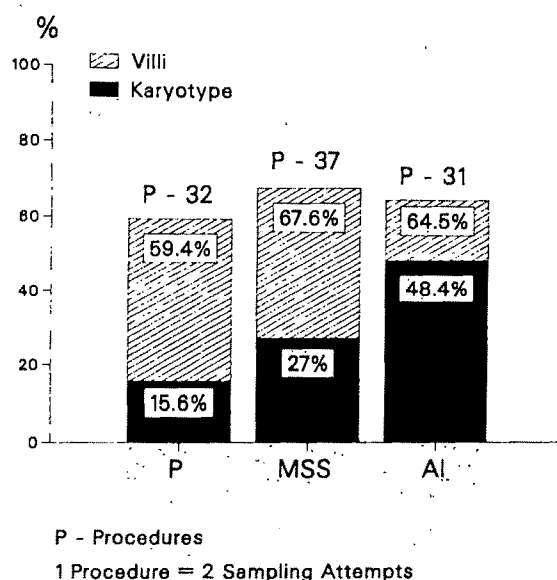
cedure. This random allocation was repeated for 100 procedures. Each patient had two procedures carried out. Therefore the first patient was assigned the first two cannulas and the second patient the next two cannulas, and so on. Thus, in the majority of patients, two different types of cannulas were used but in a proportion of patients (38%) the random allocation resulted in two cannulas of the same type being used on a single patient: the Portex Trophocan in three of fifty patients, the malleable stainless steel cannula in eight of fifty patients, and the aluminum cannula in eight of fifty patients. The random allocation of a cannula type to each procedure resulted in the Portex Trophocan cannula being used in 15 first procedures and 17 second procedures, the malleable stainless steel cannula was used in 20 first procedures and 17 second procedures, and the aluminum cannula was used in 15 first procedures and 16 second procedures. The variation in the distribution of cannula types between the first and second procedures was not significant. ( $F = 1.47$ ,  $p > 0.05$ ).

Each patient had ultrasonography performed after sampling, and the viability of the pregnancy and state of the sampling site were noted.

**Cytogenetic analysis.** The weight of the villi obtained for each sample was assessed by the cytogeneticist with reference to samples of known wet weight. Three weights were recorded: <5 mg, 5 to 10 mg, and > 10 mg.

The samples of villi were sent to one of two cytogenetic laboratories and treated by the overnight culture method described by Simoni et al.<sup>3</sup> or by a direct method as described by Burgoyne.<sup>4</sup> Improved chromosome morphologic features were obtained by fixing the villi at least overnight. Standard G-banding techniques were used. The cytogenetic sampling was deemed to be adequate if a karyotype was obtained from a minimum of two banded mitoses.





	Chi Squared	p
Villus Recovery	0.5	$p > 0.05$
Karyotype Success	8.3	$0.05 > p > 0.01$

Fig. 2. Villus recovery and karyotype success.

Table II. Patient age, gravidity, and gestation

Characteristic	Cannula		
	Portex Trophocan	Malleable stainless steel	Aluminum
Age distribution (yr)			
Mean	24.0	25.0	24.2
SD	5.4	6.2	5.7
Gravidity			
Mean	1.4	1.1	1.4
SD	1.2	1.0	1.2
Gestation (wk)			
Mean	9.1	9.3	9.7
SD	0.7	0.9	0.9

**Data recorded.** The following data were recorded by the operator: parity, length of gestation by dates, length of gestation by ultrasound estimation, and placental position. Patient reaction to the procedure was graded arbitrarily by the operator from 0 to 3. Zero indicated that no discomfort was recorded and 3 was pain such that the procedure was abandoned. Difficulty in passing the cannula through the cervix was recorded on a scale of 0 to 3 with 0 being no difficulty and 3 being the added use of a vulsella. Bleeding was graded as none, spotting, and fresh bleeding. This referred to bleeding during or immediately after sampling. Visualization of the cannula (by the operator) on the ultrasound screen during the procedure was recorded as good, poor, or none. In practice, however, in no case was the cannula not seen.

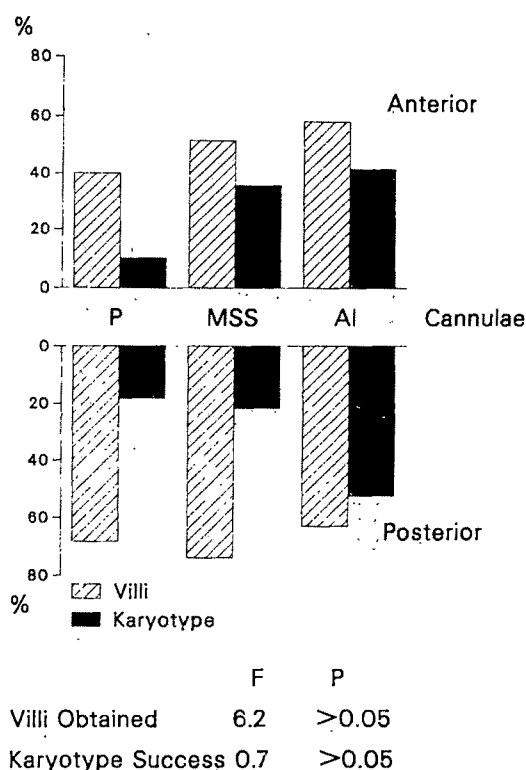


Fig. 3. Villi and placental site.

Table III. Success of chorionic villus sampling per procedure

	First procedure	Second procedure	$\chi^2$	p
Success in obtaining villi (%)	60	68	0.7	$0.5 > p > 0.25$
Karyotype success (%)	32	28	0.2	$0.75 > p > 0.5$

## Results

Table II shows the age, gravidity, and gestational age as calculated from crown-rump length measurement and indicates that there is no difference in the groups of patients assigned to each cannula when these criteria are considered. Each patient is represented twice as two procedures were carried out per patient. The success of a second procedure may have been influenced by the outcome of the first procedure. However, on examination, the success rates in obtaining villi and in obtaining a karyotype in a first or second procedure irrespective of the cannula used were not significantly different (Table III).

Fig. 2 shows the villus recovery rate and the proportion of procedures in which karyotypes were obtained for the three cannulas. Villus recovery (all weights) was greatest with the stainless steel cannula so that villi were obtained 68% of the time with two passes of a cannula. However, the differences between the

**Table IV.** Karyotype success for different quantities of villi obtained per procedure

Cannula	Quantity of villi					
	<5 mg		5-10 mg		>10 mg	
	% Success	n	% Success	n	% Success	n
Portex Trophocan	10.0	10	0.0	5	100.0	4
Malleable stainless steel	33.3	12	33.3	3	50.0	10
Aluminum	25.0	4	100.0	5	81.8	11
Total	23.1	26	46.2	13	72.0	25

n = Number of procedures in which villi were obtained.

**Table V.** Villus recovery by weight

Cannula	No villi (%)	% of procedures with villi obtained			Total No. of procedures
		<5 mg of villi	5-10 mg of villi	>10 mg of villi	
Portex Trophocan	40.4	31.5	15.6	12.5	32
Malleable stainless steel	32.5	32.4	8.1	27.0	37
Aluminum	35.5	12.9	16.1	35.5	31
Total	36.0	26.0	13.0	25.0	100

**Table VI.** Parity and chorionic villus sampling success

	No. of previous births				
	0	1	2	3	4
% Procedures with villi obtained	66.7	75.0	63.3	75.0	50.0
% Procedures with karyotype obtained	36.6	35.0	20.0	62.5	16.7
Total No. of procedures	30	20	30	8	6

three cannulas in successful villus recovery were not significantly different at the 95% level. When the proportion of karyotypes obtained is considered the aluminum cannula appears to be most effective and the Portex cannula the least, and this difference is significant ( $0.05 > p > 0.01$ ; Fig. 2). It has been reported previously that 10 mg of villus material is required for reliable karyotyping and this is confirmed by the present study for each of the three cannulas (Table IV). When the quantity of villi obtained by each cannula per procedure is examined, it can be seen that the metal cannulas are comparable and obtain >10 mg of tissue in approximately 27% to 35% of procedures. The Portex cannula in contrast only obtains >10 mg of villi in 12.5% of procedures (Table V).

Two main factors that may be expected to influence chorionic villus sampling are placental site and parity. The difference in villus recovery by placental site is not significant, but there is a 16% difference in the mean villus recovery rate depending on the sampling site (Fig. 3). Although we expected a poorer recovery rate of villi and hence reduced karyotype success in nulliparous patients, no such difference was observed (Table VI).

The ease of insertion of the cannulas, where no difficulty was encountered, is seen in Table VII. There was a highly significant difference in the ease with which the metal cannulas and especially the aluminum cannula could be inserted compared with the Portex cannula.

The ability to continuously visualize the cannula tip on the ultrasound screen was recorded for each cannula by the operator (Table VIII). Visualization of the malleable stainless steel cannula was best overall, with good visualization being recorded in 83% of procedures, compared with 72% with the Portex cannula, reflecting the enhanced ultrasound image of the olive tip, but the statistical differences between the three cannulas were not significant.

Pain and bleeding during cannula insertion are recorded in Table IX, and although there was a significant difference in discomfort with the Portex cannula compared with the metal cannulas, there was no significant difference in the bloodless sampling rates for all procedures.

The aluminum cannula proved to be best overall in villus recovery and subsequent karyotyping. This could be a reflection of the negative pressure developed along



**Table VII.** Ease of insertion of cannula into cervix

<i>Cannula</i>	<i>No difficulty (%)</i>	<i>Total No. of procedures</i>
Portex Trophocan	46.7	35
Malleable stainless steel	71.4	31
Aluminum	83.9	30

$$\chi^2 = 10.0; p = 0.01 > p > 0.001.$$

**Table VIII.** Visualization of cannula

<i>Cannula</i>	<i>% Good visualization</i>	<i>Total No. of procedures</i>
Portex Trophocan	70.0	30
Malleable stainless steel	82.8	29
Aluminum	72.0	25

$$\chi^2 = 3.1; p = p > 0.05.$$

**Table IX.** Procedures in which pain or bleeding was observed

<i>Cannula</i>	<i>% Pain score of 0</i>	<i>% Bleeding score of 0</i>	<i>No. of procedures</i>
Portex Trophocan	62.5	90.6	32
Malleable stainless steel	80.0	80.0	35
Aluminum	93.5	87.1	31
$\chi^2$	9.1	1.6	
p	0.05 > p > 0.01	p > 0.05	

**Table X.** Suction pressures of cannulas

Cannula	Pressure (mm Hg)				
	5 ml Syringe		10 ml Syringe		Dead space volume (ml)
	Mean	SE	Mean	SE	
Portex Trophocan	-625.00	0.32	-671.40	0.25	0.6
Malleable stainless steel	-613.60	0.51	-671.40	0.25	0.7
Aluminum	-597.40	0.25	-659.40	0.25	0.8

the cannula when 10 cm<sup>3</sup> of suction is applied. To investigate this, we set up a study wherein each cannula was linked via a three-way tap to a pressure transducer (Sensym LX0503A with a small dead space volume). Each cannula was tested five times. The results can be seen in Table X. The Portex and malleable stainless steel cannulas in fact have a higher negative pressure than the aluminum cannula and this may lead to fragmentation and destruction of villi more often than with the aluminum cannula.

### Comment

In this study we have demonstrated that transcervical chorionic villus sampling can be achieved by a single operator, using no accessory instrumentation on the cervix. A simple method of chorionic villus sampling is important if the cost of the procedure is to be kept to a minimum.

Our results have shown that a cannula could be passed through the cervix into the uterus without pain in 93% of patients, 30% of whom were nulliparous. In none of the 50 pregnancies studied did the method of chorionic villus sampling lead to spontaneous abortion up to the time of the termination of pregnancy.

The aluminum cannula produced the best karyotype results, reflecting the better quantity of villi per sample,

and the efficacy of any instrument used to obtain chorionic villi should be judged on the frequency with which  $\geq 10$  mg of villi is obtained for a given number of passages of an instrument.

Although many authors report villus recovery rates of up to 90% with transcervical aspiration,<sup>5</sup> they do not usually quote the karyotype success per passage of the cannula and therefore in our experience do not provide the overall villus recovery rate for amounts <10 mg. Furthermore the number of insertions of the instrument required before villi are obtained varies from one to six in reported series.<sup>6</sup> In the method described in this study villus recovery rates were no better than 70% overall when a cannula was passed twice. In those patients who after randomization underwent sampling with the same cannula for both procedures (four samples), the recovery rate remained constant at 89% for all cannulas when a posterior placental site was sampled. Thus, when counseling patients about the ability to obtain a sample by this method, we state that in 10% of cases no sample will be obtained even when the placental site is at the most favorable position for sampling.

Most reported series do not state the sampling site when the ability to recover chorionic villi with a particular instrument is analyzed,<sup>7,8</sup> but we have shown that with placentas in an anterior site the karyotype

success rate is substantially reduced over that from placentas in a posterior site, and this is especially so for the plastic Portex cannula. This could be due to the loss of ante flexion of the plastic cannula after removal of the aluminum obturator when anterior placentas are sampled.

The differing villus recovery rates for posterior versus anterior placental sites has led us to develop a transabdominal method of sampling similar to that of Smidt-Jensen and Hahnemann<sup>9</sup> for those pregnancies where the placental site is fundal and anterior to try and improve on the villus recovery rate.

When any biopsy instrument is guided under real-time ultrasound control through tissue, there still remains the problem of positive visualization of the tip of the instrument (Special topic workshop, British Medical Ultrasound Society, British Institute of Radiology, London, 1985). Although the malleable stainless steel cannula with the olive tip was visualized best, the bleeding it produced was greater than that with the other cannulas. This is probably because of the greater tip diameter compared with those of the other cannulas. Further work is being carried out on this image enhancement problem with the use of ultrasound for both transcervical and transabdominal instruments.

We wish to thank Dr. W. Main and staff, Calthorpe Nursing Home, Birmingham, England, for their assistance.

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## Endometriosis in association with uterine anomaly

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Endometriosis is frequently a chronic process, which may begin soon after menarche. The process may be enhanced by mechanical obstruction. Theories of retrograde menstruation and metaplasia still remain in vogue. Endometriosis is a cause of both acute and chronic pelvic pain in the adolescent. We present case reports of müllerian lateral wall fusion defects with surgical correction and evidence for resorption of endometriosis. Clinicians must be aware that patients with uterine anomalies may develop extensive endometriosis, which upon creation of an unobstructed outflow tract results in complete resorption. Furthermore, the mechanism of formation of endometriosis in association with an outflow tract obstruction may be very different from that associated with infertility. We recommend consideration of endometriosis and/or a reproductive tract abnormality in the adolescent with persistent pelvic pain. (*AM J OBSTET GYNECOL* 1986;154:39-43.)

**Key words:** Endometriosis, hematocolpos, uterus

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Endometriosis diagnosed in the adolescent soon after menarche may be associated with a gastrointestinal, genitourinary, or reproductive tract anomaly.<sup>1</sup> Uterus didelphys with unilateral imperforate vagina is a rare congenital anomaly which may be associated with endometriosis. The müllerian developmental abnormality



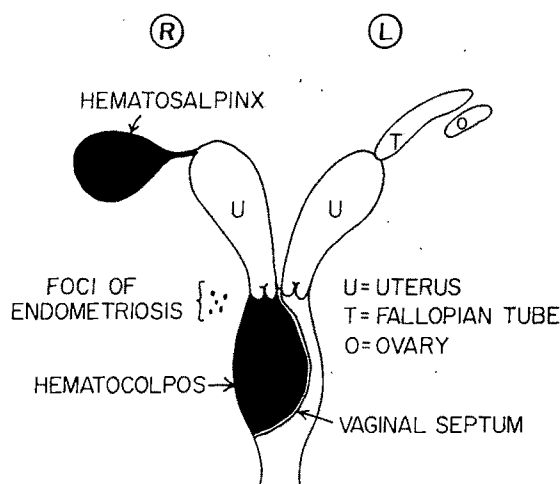


Fig. 1. Schematic drawing of diagnostic laparoscopic findings (Case 1).

is thought to be associated with formation of a partial vaginal septum, preventing one horn of the uterus from having an outlet for expulsion of endometrial desquamation products.

In 1976, Gilliland and Dyck,<sup>2</sup> in a review of the literature, found 34 cases of uterus didelphys with unilateral imperforate vagina. They added two cases from their own patient population. To date, a total of 42 reported cases of this entity has been reported.<sup>3-6</sup>

Three cases of uterus didelphys with an associated blind pouch of the vagina are presented.

### Case report

**Case No. 1.** R. T., a 12-year-old white female adolescent, was admitted to Kosair Children's Hospital, Louisville, Kentucky, on December 12, 1978. Menarche was at 10 years of age, with a second menstrual flow 1 month later. Both menses were characterized by 3 days of vaginal spotting. The second flow was followed by a sudden onset of right lower-quadrant pain, 2½ weeks prior to admission, which persisted with intermittent acute exacerbations. There were no associated genitourinary complaints. Review of systems was noncontributory. Past medical history included an appendectomy. Medications were denied.

A 17-year-old sister was noted to have "normal" cyclic menses, characterized by severe dysmenorrhea. The patient's mother was also noted to have had severe dysmenorrhea until after her first pregnancy.

Pertinent findings on physical examination included breasts consistent with Tanner Stage III and an abdomen without palpable masses. Tenderness was noted in the right lower quadrant of the abdomen, but no rebound could be elicited; rectal examination revealed a midline structure consistent with a uterus and a mass on the right. Pelvic examination revealed the vagina to have a transverse band immediately adjacent to a nulliparous-appearing cervix. The uterus was fixed and retroverted with a mass palpable in the cul-de-sac, extending to the right adnexa.

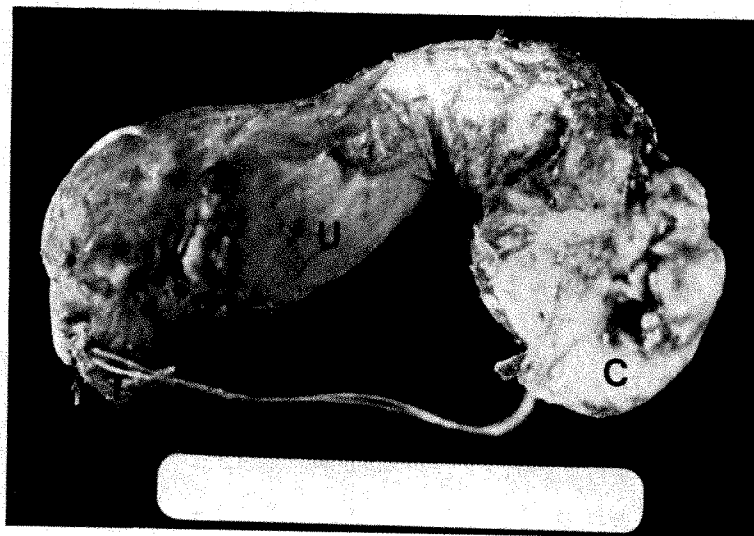
Intravenous pyelogram and barium enema studies were normal. On December 18, 1978, examination with the patient under anesthesia, vaginoscopy, laparoscopy, and colpotomy with creation of a vaginal window into an apparent hematocolpos were performed. At that time, vaginoscopy revealed the presence of a second cervix within a blind pouch of the vagina, its os (cervical) being flush with the vaginal vault. The diagnostic laparoscopy revealed extensive unilateral (right) endometriosis consistent with Stage IV (American Fertility Society classification). Fig. 1 illustrates the laparoscopic findings.

The vaginal window subsequently closed, and the patient again presented with acute abdominal pain, on April 23, 1979, of a "cyclic" nature. She was readmitted for creation of a second vaginal window April 24. In an effort to prevent closure, dilatation of the window was carried out on May 4. The vaginal window again closed, and the patient underwent exploratory laparotomy on March 10, 1980, for removal of the right uterine horn and cervix, fallopian tube, and ovary and the creation of a vaginal cuff for drainage. There was no evidence of endometriosis at laparotomy (Fig. 2). Histopathologic assessment of the pelvic organs that were removed revealed a right rudimentary horn consistent with a didelphys uterus with endocervicitis, endometritis, and acute adnexal inflammation.

The patient was discharged on the sixth postoperative day. The only complication was significant postoperative vaginal bleeding, necessitating transfusion. In addition, the patient had an infected hematoma and fever, which required antibiotic therapy (penicillin and tobramycin). After initiation of therapy there was rapid defervescence within 48 hours. One year after operation the patient was doing well.

**Case No. 2.** S. C., a 12-year-old white female adolescent, presented with an abdominal mass and a past history of hematocolpos and hematometra in association with a didelphys uterus. Menarche occurred at 11 years of age; three subsequent "normal" menstrual periods followed by cyclic abdominal pain at the time of "expected menstruation." An intravenous pyelogram revealed a normal left renal ureteral system and non-functioning right collecting system.

On September 24, 1983, examination with the patient under anesthesia demonstrated a 12-week-size pelvic mass. A right hemivaginal "bulge" with two cervices was noted. The patient underwent excision of the vaginal septum with drainage of the hematocolpos. Subsequently a fever (101.5° F) necessitated readmission on October 10. Parenteral antibiotics (penicillin and tobramycin) were initiated. She was subsequently brought to the operating room because of a rapidly deteriorating clinical course that necessitated operative intervention. Diagnostic laparoscopy revealed a didelphys uterus, with the right side 6 cm longer than the left, and a right adnexal mass involving the omentum and bowel. There was no clearly identifiable fallopian tube or ovary on the affected side. A catheter was placed into the right cervix and uterus, with drainage of 40 ml of purulent material. Exploratory laparotomy



**Fig. 2.** Rudimentary horn consistent with a didelphic uterus with endocervicitis, endometritis, and acute adnexal inflammation. There was no evidence of endometriosis at the time of exploratory laparotomy (Case 1). C = Cervical; U = uterine horn; T = fallopian tube.

was performed, at which time a right tuboovarian mass measuring 13.5 by 8.6 by 8.4 cm was removed. The left tube and ovary as well as the left hemiuterus appeared normal. The intraoperative procedure included excision of one focus of fibrous connective tissue, containing hemosiderin macrophages (probable endometriosis), over the bladder flap. The patient's clinical course was then characterized by defervescence. She was discharged home on the sixth postoperative day.

**Case No. 3.** S. S., a 12-year-old white female adolescent, had menarche that began at 11 years, 9 months of age. Menses were noted to be infrequent and associated with cyclic abdominal pain. An exploratory laparotomy by her referring physician demonstrated an "expanding pelvic mass"; a didelphic uterus with hematocolpos was noted. The patient was referred to the University of Louisville for treatment.

The pertinent findings on physical examination were confined to the pelvis where a protruding blind vaginal pouch with a 5 cm right pelvic mass was noted. At the left apex of the vagina a cervix was noted.

A preoperative intravenous pyelogram revealed absence of the right kidney and a normal left kidney and collecting system. On the third day of hospitalization resection of the blind vaginal pouch and drainage of the hematocolpos were performed. There was a rapid decrease in the size of the mass. Visualization of the right cervix was then accomplished. After operation, the patient had an uneventful course; prophylactic parenteral antibiotics were administered.

#### **Comment**

Endometriosis is frequently a chronic process, felt to begin at menarche and enhanced by mechanical obstruction (imperforate vaginal septum or congenital atresia of the cervix).<sup>8</sup> Fallon et al.<sup>9</sup> hypothesized that endometriosis tends to develop after 5 or more years of continued menses without pregnancy.

The mechanism of development of endometriosis can be associated with a reproductive organ abnormality. In the absence of such an anomaly, theories of retrograde menstruation<sup>10</sup> and a totipotential mesothelium undergoing a metaplastic conversion to functional endometrium as a result of recurring menstrual insults<sup>11</sup> may account for its development. Endometriosis has been noted to be a common cause of recurrent pelvic pain in the adolescent.<sup>7</sup> Table I includes a literature review of endometriosis as reported in the adolescent.

The genetic aspects of endometriosis remain an interesting question. Simpson et al.<sup>12</sup> postulated a polygenic/multifactorial pattern of inheritance with a 6.9% recurrence rate for first-degree relatives. In addition to genetic patterns, other predisposing factors to endometriosis include menorrhagia, imperforate hymen, and other vaginal wall abnormalities. Endometriosis is commonly found in association with female infertility. The exact relationship between endometriosis and infertility is not well understood. Alterations in prostaglandin content of peritoneal fluid<sup>13</sup> as well as macrophage content<sup>14</sup> have been hypothesized.

We reported here three cases of uterus didelphys with unilateral imperforate vagina. Two were associated with endometriosis, and the third did not undergo laparoscopy or laparotomy. In Case 1 complete remission of endometriosis was demonstrated after a path was provided for efflux of menstruum. In Case 2 a focus of endometriosis was noted.

Uterus didelphys is thought to be associated with a lack of müllerian duct fusion, which is normally completed by the sixteenth week of gestation. Wiersma et al.<sup>15</sup> speculate that the partial vaginal septum is the result of an abnormality in the development of the



**Table I.** Endometriosis in adolescents

<i>Series*</i>	<i>Description</i>	<i>Series</i>	<i>Description</i>
Schiffrin et al.	Endometriosis in 15 cases of patients 12 to 20 yr old, presenting with epigastric pain, lower-quadrant abdominal pain, or dysmenorrhea. There was an associated increased incidence of gastrointestinal and genitourinary anomalies.	Bruser	Appendectomy with inability to relieve symptoms, 12 patients, 19 to 31 yr old. Subsequently each was found to have endometriosis
Fallon	4% of teenage girls (9 of 225) had endometriosis at the time of preoperative diagnosis of acute appendicitis	Depp and Pope	Of 233 patients between 10 and 19 yr old, 2.3% had endometriosis
Derryberry and Bonney	Endometrioma in 15-year-old black teenager with normal vagina and introitus and stenotic cervical isthmus	TeLinde	Of 8789 pelvic laparotomies, 1.2% had microscopically diagnosed endometriosis within 10 yr of menarche
Bullock et al.	Symptomatic endometriosis in 4 teenagers, 17 to 19 yr old, Wilford Hall USAF Medical Center	Sutton	Girl 14 yr old with imperforate hymen, hematocolpos, hematometra (bilateral hematosalpinges and peritoneal implants)
Meigs	Endometrial cysts in adolescents 13 to 19 yr old	Sutton	case of reflux menstruation with bicornuate uterus. Intense distended left horn
Hanton et al.	68 young patients (63 with menarche 5 to 10 yr before diagnosis and 6 with congenital obstruction of menstrual flow) in 30 yr review of Mayo Clinic experience	McDonald	Endometriosis associated with didelphic uterus
Hanton et al.	Case report of endometriosis associated with complete or partial obstruction of menstrual egress	Mittal et al.	Endometriosis of appendix, presenting as acute appendicitis. Patients were 14 to 62 yr old. 40% (50 patients) incidence of gastrointestinal tract involvement with endometriosis
Moore et al.	7 of 127 patients with ovarian masses had endometriosis. Age range birth to 17 yr	King	Metastatic endometriosis in abdominal scars of 17 yr old girl. Pain and tenderness with right lower-quadrant abdominal pain noted and preoperative diagnosis of acute appendicitis

\*These references will be provided to the reader on request.

sinovaginal bulb.<sup>15</sup> It is also known that this congenital anomaly is nearly always associated with renal agenesis on the affected side.<sup>1</sup> These cases offer presumptive evidence of the role of retrograde menstruation in the histogenesis of endometriosis and demonstrate a spontaneous and complete regression of endometriosis after the creation of an outlet for the menstrual effluvium away from the peritoneal cavity (Case 1). If this retrograde flow is stopped early enough, endometriosis might be prevented or, if found, reversed with appropriate anatomic correction. This leads to a second assumption, that there is normally a mechanism, perhaps immunologic, that would prevent implantation of endometrial tissue, since it is known that most patients have endometrial fragments in the peritoneal cavity after diagnostic procedures such as dilatation and curettage.<sup>16</sup> Perhaps, in the patient who develops endometriosis, this protective mechanism is overwhelmed by the continuous and chronic barrage of endometrial reflux, thus allowing the seeding of the peritoneum and formation of endometriosis.

It is implied that creation of a vaginal window was associated with menstrual outflow. Provision of such a tract apparently reversed extensive pelvic cavity endometriosis including unilateral endometrioma for-

mation (Case 1), which probably evolved over a period of several years from menarche to diagnosis via laparoscopy.

A didelphic uterus can be surgically corrected by lateral anastomosis of the unobstructed horn to the obstructed horn. Details of treatment alternatives are addressed by Jones.<sup>17</sup>

We speculate that endometriosis and endometrioma formation in association with hematocolpos are different entities from those found in the absence of a uterine or a vaginal anomaly. Furthermore, chronic pelvic pain in the adolescent should be carefully evaluated; where indicated, assessment should include diagnostic laparoscopy to provide early diagnosis of endometriosis with or without a uterine anomaly and subsequent medical therapy.

Two patients underwent diagnostic laparoscopy that revealed endometriosis. In all three, a vaginal window into the blind pouch was created. Laparotomy after the laparoscopic diagnosis of endometriosis and creation of a vaginal window revealed no evidence of endometriosis in Case 1 and a 1 mm lesion in Case 2. In Case 3 neither laparoscopy nor laparotomy was done.

Clinicians are now aware of endometriosis occurring

in 47% of adolescents evaluated for chronic pelvic pain.<sup>7</sup> We recommend consideration of endometriosis and/or a reproductive tract abnormality in the adolescent with persistent pelvic pain. We believe we are the first to report cases of uterus didelphys with unilateral imperforate vagina associated with endometriosis in which extensive endometriosis was noted to be in complete remission after creation of a vaginal window. We again emphasize that chronic pelvic pain in the adolescent should be carefully evaluated; when indicated, assessment should include diagnostic laparoscopy to provide early diagnosis of endometriosis with or without a uterine anomaly and subsequent medical therapy in an effort to prevent significant compromise of fertility. This implies a different mechanism of endometriosis formation from that more commonly noted with infertile women.

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# Assessment of uterine activity in ambulatory patients at high risk of preterm labor and delivery

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With use of an ambulatory tocodynamometer, prelabor uterine activity was recorded daily during pregnancy in 34 patients at increased risk of preterm labor and delivery. The data indicate that the frequency of contractions was significantly greater among women who subsequently developed preterm labor when compared to that observed among women who labored at term. This difference was present up to several weeks before the onset of labor. An additional significant rise in frequency of contractions could be observed within the last 24 hours before the development of clinically apparent preterm labor, as evidenced by progressive cervical dilation and effacement. If these results can be reproduced in a larger sample, intermittent ambulatory monitoring may offer an effective way to better identify patients at risk of preterm labor as well as enhance the early diagnosis of this event. (AM J OBSTET GYNECOL 1986;154:44-7.)

**Key words:** Ambulatory monitoring, preterm labor

Most of the available information concerning the frequency of prelabor uterine contractions was obtained from pregnant subjects who were either in recumbency or otherwise limited in their activities.<sup>1-8</sup> However, since the great majority of women are physically active during pregnancy, the reported data may not accurately reflect the true characteristics of prelabor uterine activity and therefore be limited in their clinical usefulness. Determining normal versus abnormal uterine activity could be of particular significance for the management of those patients who are at high risk of preterm labor and delivery.

In the following study, uterine activity was monitored daily in a group of fully ambulatory patients who were at high risk of preterm labor and delivery. The results indicate that significant differences exist in the frequency of prelabor uterine contractions between those patients whose labor begins at term and those who developed preterm labor. Since the differences in uterine activity between these groups of patients were often apparent before the development of preterm labor, it is suggested that ambulatory tocodynamometry may be instrumental in enhancing the early detection of preterm labor.

## Material and methods

Thirty-four Caucasian women, 17 of whom developed labor before 35 gestational weeks (group A) and 17 of whom labored after 36 weeks' gestation (group B), were included in this study. The risk indicators for preterm labor in each study group are given in Table 1. This patient sample represents a group of 34 consecutive patients who underwent home monitoring and fulfilled the following criteria: (1) labor and delivery were spontaneous (no intentional deliveries because of maternal or fetal indications), (2) patients were not instructed to limit their daily activities, and (3) they received no prophylactic uterine relaxants.

Each patient received a recently developed tocodynamometry device designed for ambulatory monitoring and storage of uterine activity data.<sup>9\*</sup> After monitoring, the stored data were transmitted at a convenient time via the telephone to a receiving tocograph located in the study center. Average transmission time was 6 to 8 minutes for a 200-minute segment of stored data. A description of the TermGuard device, its dimensions, reliability, and mode of operation were previously reported in detail.<sup>9</sup> Briefly stated, a lightweight (383 gm) recording and transmission device, which can be carried on a belt or hung from a shoulder strap, was used. This device allows free ambulation without interference. The tocodynamometer sensor was designed to take advantage of the Guard-Ring principle as described by Smyth<sup>10</sup> and Bell.<sup>11</sup> Previous experience has shown this design to be particularly reliable in record-

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**Table I.** Clinical characteristics of study groups (mean  $\pm$  SD)

	Maternal age (years)	Nulliparous/ multiparous ratio	Multifetal gestation	Previous preterm birth	Other factor*
Group A, preterm labor (n = 17)	30.1 $\pm$ 4.4	8:9	8	7	6
Group B, term labor (n = 17)	31.9 $\pm$ 4.4	8:9	5	7	7

\*Diethylstilbestrol uterine anomaly, cerclage, etc.

ings of mild contractions; which are predominant during the prelabor period.<sup>7,9</sup>

Uterine activity was recorded four times daily for a cumulative total of at least 200 min/day. Included were monitoring periods representative of the morning, afternoon, and evening hours. While all women were free to continue with their normal daily activities, recordings were done mostly in the upright position (sitting or standing). Less than 1% of the data was obtained from subjects who were walking at the time of monitoring. There were no restrictions on sexual activity, but only those of the patients who developed preterm labor (group A) were routinely questioned about this subject on admission. However, since only one patient in this group reported having intercourse within 24 hours of developing preterm labor, the issue of sexual activity was not pursued in this study. Outpatient prenatal care was provided every 1 to 3 weeks and included a pelvic examination for evaluation of cervical status. In group A (preterm labor) one patient received treatment for a positive group B  $\beta$ -streptococcus cervical culture, and in group B (term labor) two patients were treated for symptomatic monilial vulvovaginitis. There were no positive urinary cultures in either group.

The daily records of each patient were carefully reviewed, and only contractions of  $>35$  seconds were included in the analysis. Contractions of  $<35$  seconds, most of which were of the low-amplitude high-frequency type ("Alvarez waves") were excluded from the current analysis. The frequency of contractions was averaged for each gestational week. Length of pregnancy was determined by use of the date of the last menstrual period and ultrasonography and was later confirmed by neonatal evaluation. For those patients who developed preterm labor, only recordings that preceded the admission and treatment for preterm labor were used. The following criteria were required before the diagnosis of preterm labor was made: (1) gestational age of  $<35$  weeks' gestation; (2) normal living fetus(es); (3) intact membranes; (4) persistent uterine contractions occurring at least every 10 minutes for 1 hour and not responding to bed rest, hydration, or sedation; and (5) documentation of progressive cervical dilation and/or effacement. Patients were never treated for preterm labor based solely on the frequency of uterine activity

**Table II.** Ambulatory monitoring intervals (mean  $\pm$  SD)

	Gestational week at start	Gestational week at completion	Duration (wk)
Group A, preterm labor	24.5 $\pm$ 3	29.2 $\pm$ 3.6	4.5 $\pm$ 3.5
Group B, term labor	25.6 $\pm$ 3.3	34.5 $\pm$ 2.7	8.9 $\pm$ 3

unless this activity was associated with cervical changes. Sequential comparisons between the mean weekly frequencies of uterine activity within the same study group were done by analysis of variance for repeated measures. Comparisons of the mean weekly frequency of uterine activity between the study groups at any particular gestational age were done by Student's *t* test for unpaired variables. The results are expressed as means  $\pm$  SD.

## Results

The mean gestational age at the onset of ambulatory recording and the duration of monitoring for each of the groups are given in Table II. It is evident that monitoring of several group A patients was discontinued earlier, since these patients developed preterm labor, and once tocolytic therapy was started, their data were excluded from analysis.

The mean number of contractions per hour in relation to gestational age for each of the two groups is given in Fig. 1. The differences between the groups' mean frequency of contractions were significant and particularly pronounced after 30 weeks' gestation. Analyses of the differences from 1 week to the next within the same group did not demonstrate significant changes. However, as pregnancy advanced, there was a definite trend toward an increase in frequency of contractions.

The mean frequency of uterine activity as it relates to the number of weeks before preterm labor (for group A) or before 37 weeks' gestation (for group B) is provided in Fig. 2. A significant difference in the frequency of contractions between the group that developed preterm labor and the one that did not was evident several weeks before labor. However, comparison of the mean frequency from 1 week to the next



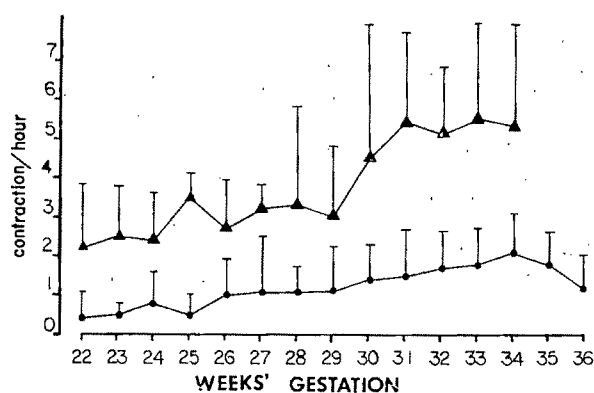


Fig. 1. Mean frequency of contractions during pregnancy in patients having preterm labor (▲) and patients having term labor (●). Mean  $\pm$  SD.

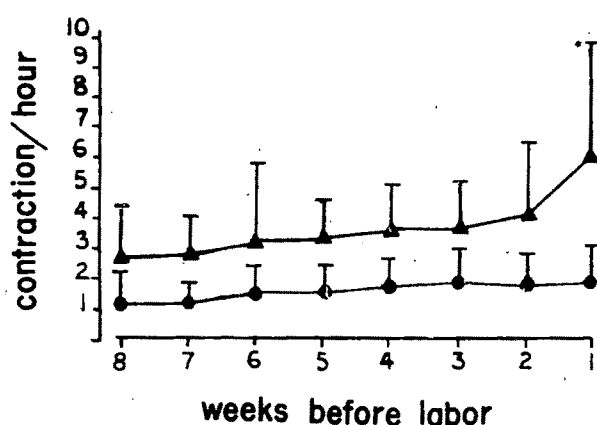


Fig. 2. Mean frequency of contractions during the last 8 weeks before preterm labor (▲) and before 37 weeks' gestation (●). Mean  $\pm$  SD. \* =  $p < 0.005$ , last week versus preceding weeks.

within the same study group did not disclose any significant differences except for a significant increase during the week in which preterm labor developed. For the final 7 days before the development of preterm labor, a more detailed, daily rather than weekly, analysis of uterine activity was performed and is shown in Fig. 3. There was a sudden and significant increase in frequency of uterine contractions during the last 24 hours before the appearance of progressive cervical changes (that is, preterm labor).

#### Comment

Previous reports indicated that intrapartum ambulation was accompanied by a significant increase in uterine activity.<sup>12, 13</sup> It was therefore expected that the antepartum uterine activity data obtained in this study of ambulatory patients would also be different from that found in recumbent patients.<sup>1-7</sup> However, the frequency of contractions in the current study seemed to be comparable to that reported by several other investigators

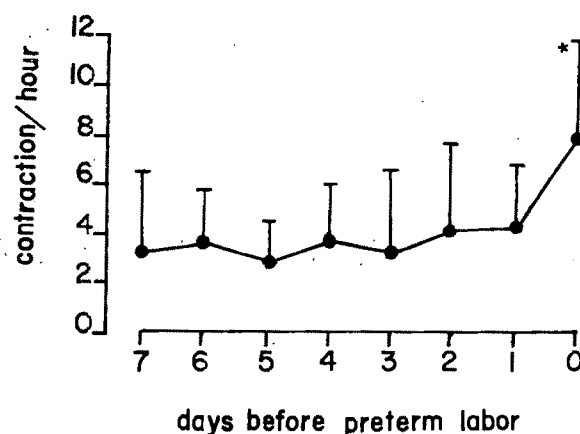


Fig. 3. Mean frequency of contractions during the last 7 days before preterm labor. Mean  $\pm$  SD. \* =  $p < 0.001$ , last days versus preceding days.

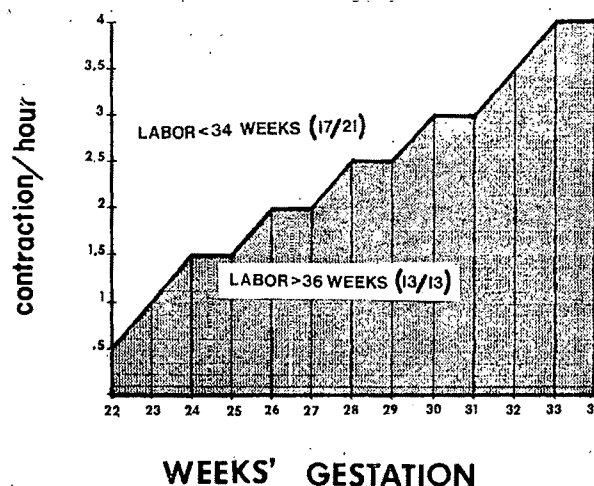


Fig. 4. Calculated mean frequency of contractions representing the best fitting separation between patients who did and those who did not experience preterm labor.

who monitored prelabor uterine contractions by using conventional stationary monitors.<sup>1-8</sup> Furthermore, the suggestion that patients who are destined to develop preterm labor manifest increased prelabor uterine activity when compared to those patients who begin labor at term<sup>4, 7</sup> was confirmed by us in ambulatory patients. Although the differences in uterine activity that were reported by others could be confounded by the comparison of groups who did not have an even distribution of risk factors,<sup>4, 7</sup> the proportions of major risk indicators in each of the current study groups were similar. It can therefore be safely assumed that in this study the only major variable that could be associated with the early significant increase in uterine activity was the subsequent development of preterm labor.

To evaluate the potential clinical value of the data, the maximum mean weekly frequency of contractions

that could be considered normal was determined for each gestational age (Fig. 4). A woman whose weekly mean frequency of contractions exceeded that number had an 80% (17 of 21) likelihood of belonging to the group that ultimately experienced preterm labor. On the other hand, study patients who had a mean weekly frequency below that shown in Fig. 4, experienced labor at term. It is clear that the number of patients may be too small for firm conclusions, but a general trend can definitely be observed. Of note is the fact that almost all the patients who were in the excessive uterine activity zone, but did not have preterm labor, had multifetal gestations. This finding may indicate that these pregnancies have more uterine contractions even in the absence of preterm labor.<sup>14</sup>

Of particular importance is the ability to distinguish between the increased baseline activity that is present for up to several weeks before preterm labor (that is, cervical changes) (group A, Fig. 2) and the uterine activity that is observed during the last 24 hours and does ultimately change the cervix. This study's data seem to indicate that in addition to their significantly higher baseline contraction frequency, those patients who developed preterm labor had an approximately twofold increase in uterine activity during the last day before admission and treatment for preterm labor. Therefore, while uterine activity in excess of that shown for group A in Fig. 2 may be predictive of preterm labor, daily monitoring of the patients would still be necessary, since the changes in contraction frequency that ultimately lead to cervical changes appear only within the last 24 hours.

In summary, it is suggested that ambulatory monitoring may enhance our ability to predict who are the patients at greater risk of preterm labor as well as establish an accurate early diagnosis of preterm labor within 24 hours of its occurrence. The close surveillance of women at high risk for preterm labor should allow earlier intervention to prevent preterm birth. If these predictions are confirmed in a larger prospective investigation, a substantial reduction in the current rate

of preterm deliveries should not be considered out of reach.

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# Interrelationship between amniotic fluid C-peptide and catecholamines in the last trimester of diabetic pregnancy

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Amniotic fluid concentration and content (amniotic fluid volume  $\times$  concentration) of C-peptide and catecholamines (epinephrine, norepinephrine) and their interrelationship was studied in nine women with gestational diabetes, in 14 women with type I diabetes, and in 20 healthy control women between the thirty-sixth and thirty-ninth week of gestation. Mean amniotic fluid volume was significantly larger ( $p < 0.05$ ) in the type I diabetic group than in the control group. Mean concentration and content of amniotic fluid C-peptide were elevated in women with gestational diabetes, significantly so in women with type I diabetes ( $p < 0.05$ ) as compared with nondiabetic control women. Mean amniotic fluid catecholamine concentrations were lower, although not statistically so, in both insulin-dependent and gestational diabetic women than in control women. Mean amniotic fluid catecholamine content was higher, although not statistically so, in women with gestational diabetes than in control women. In the type I diabetic group, epinephrine content was significantly lower ( $p < 0.05$ ) and norepinephrine content significantly higher ( $p < 0.05$ ) than in the control group. A significant positive correlation between the content of norepinephrine and C-peptide was found in control women ( $r = 0.57$ ;  $p < 0.05$ ) and in women with gestational diabetes ( $r = 0.75$ ;  $p < 0.05$ ). The close interrelationship could indicate a parallel maturation of these two hormonal systems. (AM J OBSTET GYNECOL 1986;154:48-52.)

**Key words:** Amniotic fluid, C-peptide, catecholamines, diabetic pregnancy

Catecholamines in the amniotic fluid are considered to be primarily of fetal origin and to reach the amniotic cavity via fetal urine.<sup>1</sup> Since the placenta efficiently inactivates catecholamines of maternal origin by both deamination and O-methylation, only minimal amounts are transferred from the mother to the fetus.<sup>2</sup> Toward term of pregnancy the catecholamine concentrations in amniotic fluid increase significantly, suggesting a progressive maturation of the fetal sympathetic nervous system.<sup>3</sup> Fetal hypoxia, which is known to activate the fetal sympathoadrenal system,<sup>4,5</sup> may be accompanied by an increased concentration of catecholamines in the amniotic fluid. Significantly increased levels of catecholamine metabolites have been reported in amniotic fluid in pregnancies with growth-retarded fetuses.<sup>6</sup> Highly increased levels of catecholamines in amniotic fluid were seen after methadone treatment of pregnant women,<sup>7</sup> suggesting that accentuated fetal sympathetic activity may be reflected in amniotic fluid.

It has long been recognized that the fetus of the diabetic mother may suffer chronic hypoxia.<sup>8</sup> Erythro-

cytosis, increased extramedullary erythropoiesis, and, more recently, elevated plasma levels of erythropoietin<sup>9</sup> have been demonstrated in newborn infants of diabetic mothers. On the basis of experimental studies in animals, several authors<sup>9-11</sup> have proposed that impaired oxygen supply from the mother and/or fetal hyperinsulinism may be responsible for these hypoxia-compensating responses in the fetus. However, lower than normal concentrations of both norepinephrine and metanephrine, a major metabolite of catecholamines, have recently been recorded in amniotic fluid from insulin-dependent diabetic women.<sup>12,13</sup> It was hypothesized that these low concentrations could reflect decreased biosynthesis of norepinephrine and delayed maturation of the sympathetic nervous system.<sup>12</sup> A low concentration of norepinephrine in amniotic fluid could, however, also be explained by a larger amniotic fluid volume that is frequently seen in pregnancies complicated by diabetes.

The object of the present study was to evaluate the fetal C-peptide-catecholamine interrelation in pregnancies complicated by gestational or type I diabetes. Amniotic fluid C-peptide, which is considered to be entirely of fetal origin, was measured as an index of fetal insulin secretion. The study was performed before the spontaneous onset of labor in order to avoid influences on hormone concentrations by various stress factors induced during labor and delivery. In addition, amniotic fluid volumes were determined in order to assess possible dilution effects due to hydramnios.

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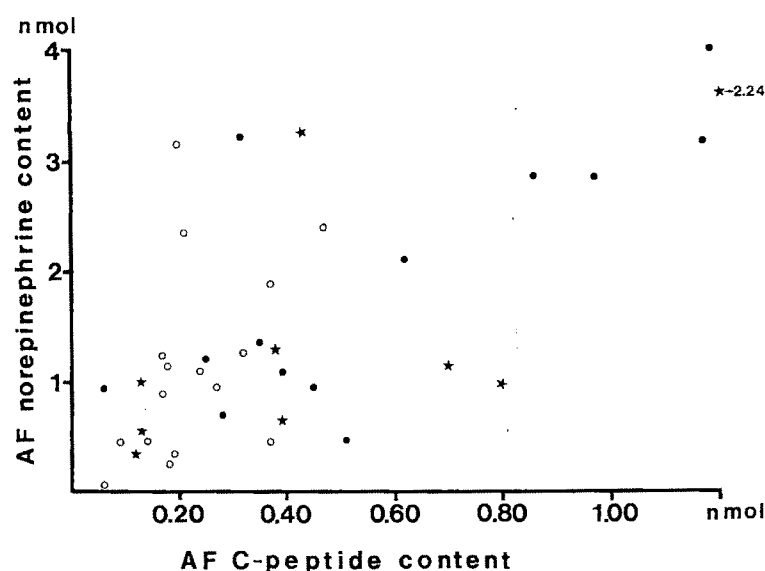


Fig. 1. Norepinephrine content values plotted against C-peptide content values in amniotic fluid. Gestational diabetic women (★):  $r = 0.75$ ,  $p < 0.05$ ; insulin-dependent diabetic women (●):  $r = 0.54$ ,  $p > 0.05$ ; normal control women (○):  $r = 0.57$ ,  $p < 0.05$ .

Table I. Clinical data of diabetic and nondiabetic mothers and their newborn infants (mean  $\pm$  SEM)

	Diabetic women		Nondiabetic control women
	Gestational	Insulin-dependent	
No. of patients	9	14	20
Mothers			
Age (yr)	31.9 $\pm$ 2.1	29.6 $\pm$ 1.2	28.0 $\pm$ 1.2
Duration of diabetes at delivery (yr)	0.3 $\pm$ 0.1	10.1 $\pm$ 2.3	—
White's class (n)	9AB	6B, 5C, 3D	—
Infants			
Route of delivery (n)			
Vaginal	7	8	12
Cesarean section	2	6	8
Birth weight (gm)	3391 $\pm$ 211	3534 $\pm$ 127	3069 $\pm$ 84
Birth length (cm)	49.5 $\pm$ 0.8	50.6 $\pm$ 0.5	49.5 $\pm$ 0.5
Birth weight (percentile)	56 $\pm$ 10	74 $\pm$ 6	38 $\pm$ 5
Gestational age (days)	272 $\pm$ 2	266 $\pm$ 2	272 $\pm$ 2

## Material and methods

**Subjects.** Twenty-three consecutive diabetic pregnancies were studied. Gestational age in this group as well as in the control group was carefully assessed from the patient's menstrual history and confirmed by clinical examination and ultrasound in early pregnancy. There were 14 women with insulin-requiring (type I) diabetes. According to the classification of White,<sup>14</sup> there were six Class B, five Class C, and three Class D patients. Nine patients had gestational diabetes and received insulin treatment during the last trimester of pregnancy. Details regarding the management of diabetic mothers during pregnancy have been given previously.<sup>15</sup> Maternal and infant characteristics are given in Table I. All infants of diabetic mothers were observed in a neonatal intensive care unit. The majority of the infants had an uneventful neonatal period. Neo-

natal complications encountered were: hypoglycemia (blood glucose below 1.7 mmol/L or 30 mg/dl),  $n = 3$ ; erythrocytosis (venous hematocrit value above 70%),  $n = 1$ ; hyperbilirubinemia (serum bilirubin above 300  $\mu$ mol/L or 17.5 mg/dl),  $n = 3$ ; and feeding problems (the early feeding schedule could not be followed and the infant required feeding by the nasogastric route and/or the infant had significant amounts of gastric residuum),  $n = 1$ .

The control group comprised 20 nondiabetic pregnant women. The control material was selected from our high-risk obstetric population and included patients with a suspicion of intrauterine growth retardation and patients undergoing repeated cesarean section or induction of labor. In order to be included as controls in the study the neonate should have an uneventful neonatal course and a birth weight within the

**Table II.** Mean  $\pm$  SEM values for gestational age at sampling, amniotic fluid C-peptide, amniotic fluid volume, and concentration and total amount of epinephrine and norepinephrine in diabetic women and nondiabetic control subjects

	Diabetic women		Nondiabetic control women	p*
	Gestational	Insulin-dependent		
No. of patients	9	14	20	
Gestational age at sampling (days)	263 $\pm$ 3	257 $\pm$ 2	261 $\pm$ 2	NS
C-peptide (nmol/L)	0.57 $\pm$ 0.12	0.58 $\pm$ 0.07	0.37 $\pm$ 0.02	<0.05
Epinephrine (nmol/L)	1.05 $\pm$ 0.33	0.57 $\pm$ 0.07	1.31 $\pm$ 0.48	NS
Norepinephrine (nmol/L)	1.64 $\pm$ 0.29	2.22 $\pm$ 0.34	2.64 $\pm$ 0.55	NS
Amniotic fluid volume (ml)	846 $\pm$ 149 (n = 9)	1022 $\pm$ 176 (n = 13)	590 $\pm$ 59 (n = 16)	<0.05
C-peptide (nmol)	0.50 $\pm$ 0.22	0.57 $\pm$ 0.10	0.23 $\pm$ 0.02	<0.01
Epinephrine (nmol)	0.90 $\pm$ 0.30	0.56 $\pm$ 0.10	0.72 $\pm$ 0.49	<0.05
Norepinephrine (nmol)	1.42 $\pm$ 0.39	1.92 $\pm$ 0.32	1.15 $\pm$ 0.22	<0.05

There were no significant differences between values of gestational and insulin-dependent diabetic women or between values of gestational diabetic and control women.

NS = Not significant.

\*p = Level of significant difference (Mann-Whitney U test) between values of insulin-dependent diabetic women and normal control women.

tenth and ninetieth percentiles for gestational age and sex according to Swedish standards<sup>16</sup> (Table I).

**Amniotic fluid samples and determination of the amniotic fluid volume.** The amniotic fluid samples were collected in connection with routine transabdominal amniocentesis for assessment of fetal lung maturity. Amniocentesis was performed in all subjects on clinical grounds between 36 and 39 weeks' gestation. Samples contaminated by blood or meconium were excluded. In connection with the amniocentesis amniotic fluid volume was determined by a dilution technique with the use of para-aminohippurate solution.<sup>17</sup> Details of the procedure were described previously.<sup>18</sup> Hydramnios was defined as an amniotic fluid volume >1500 ml. All patients gave their informed consent to participate and the study was approved by the local ethical committee.

**Analytical procedures.** The lecithin/sphingomyelin ratio was determined by a modified thin-layer chromatographic procedure according to Gluck et al.<sup>19</sup> and Björkhem et al.<sup>20</sup> Para-aminohippurate was determined by a colorimetric method.<sup>21</sup> C-peptide was measured by radioimmunoassay after samples had been treated with polyethylene glycol to remove insulin antibodies.<sup>22</sup> Catecholamines were determined by high-performance cation exchange liquid chromatography with electrochemical detection, as described and validated for plasma previously.<sup>23, 24</sup> The amniotic fluid contents of C-peptide and catecholamines were calculated by multiplying their concentration by the amniotic fluid volume.

**Statistical methods.** Mean values and SEM were computed by conventional methods. Differences between groups were tested by the Mann-Whitney U test for

unpaired variates. Covariation was tested by Spearman's non-parametric rank correlation. A p value < 0.05 was considered to be statistically significant. Results are presented as means  $\pm$  SEM.

## Results

Gestational age at the time of amniotic fluid sampling was not different between mothers with gestational or type I diabetes and control women (Table II). The mean amniotic fluid volume was significantly larger ( $p < 0.05$ ) in the type I diabetic group than in the control group. Among the type I diabetic mothers the amniotic fluid volume was >1000 ml in five and two had hydramnios. In the gestational diabetic group two had an amniotic fluid volume >1000 ml and one had hydramnios. Only one control mother had an amniotic fluid volume exceeding 1000 ml.

Both the concentration and the total amount of C-peptide in amniotic fluid were significantly higher in the type I diabetic group than in the control group ( $p < 0.05$  and  $p < 0.01$ , respectively, Table II). The corresponding mean values in the gestational diabetic group were higher than in the control group, but the difference was not significant.

Mean catecholamine concentrations were lower, although not statistically so, in insulin-dependent and gestational diabetic women compared with control women. The total amount of epinephrine was significantly lower ( $p < 0.05$ ) and the total amount of norepinephrine significantly higher ( $p < 0.05$ ) in type I diabetic women than in control women. The mean catecholamine content was higher, although not statistically so, in gestational diabetic women than in control women (Table II).



The total amount of norepinephrine and C-peptide was correlated in control women ( $r = 0.57$ ,  $n = 16$ ,  $p < 0.05$ ) and in women with gestational diabetes ( $r = 0.75$ ,  $n = 9$ ,  $p < 0.05$ ) but not significantly so in type I diabetic mothers ( $r = 0.54$ ,  $n = 13$ ,  $p > 0.05$ ) (Fig. 1). Catecholamine concentrations were not correlated with amniotic fluid volume.

### Comment

The present observation of both elevated concentration and content of amniotic fluid C-peptide in diabetic pregnancies suggesting fetal hyperinsulinism was in accordance with our previous reports.<sup>25,26</sup> The somewhat lower concentrations of norepinephrine in amniotic fluid in diabetic pregnancy was also in accordance with a recent study.<sup>12</sup> In contrast to the lower concentrations of catecholamines found in our study in insulin-dependent diabetic women, others have reported slightly, but not significantly, elevated levels of the catecholamine metabolites 4-hydroxy-3-methoxy-phenyl glycol and 4-hydroxy-3-methoxy-mandelic acid in amniotic fluid.<sup>6</sup> Differences in concentrations of hormones and metabolites between diabetic patients and between diabetic patients and control women can be partly attributed to greater than normal variation in amniotic fluid volumes between diabetic patients and the tendency to larger amniotic fluid volumes in the diabetic group. The fact that the total content of norepinephrine in amniotic fluid was somewhat higher, whereas the content of epinephrine was lower, in type I diabetic pregnancies than in the control group, must be interpreted with caution in view of absence of precise turnover data. Catecholamines are metabolized to a great extent and hence responses in fetal catecholamine secretion to various stimuli might be better reflected in amniotic fluid by 4-hydroxy-3-methoxy-phenyl glycol and 4-hydroxy-3-methoxy-mandelic acid. Still one could speculate that a low fetal epinephrine production around the time of birth in diabetic pregnancy could cause inappropriate release of surfactant and impaired absorption of lung fluid and thus contribute to an increased risk of respiratory distress in the newborn infant.<sup>27</sup> However, this speculation could not be supported by clinical data in the present study.

The differences in catecholamine levels in amniotic fluid between the three groups of type I diabetic patients, gestational diabetic patients, and control women were small and do not support the hypothesis of a delayed maturation of the sympathoadrenal system in fetuses of diabetic mothers. Neither do the data suggest the opposite, that is, that of an enhanced catecholamine secretion due to fetal hypoxia related to fetal hyperinsulinism. In the present study amniotic fluid C-peptide, which gives a measure of fetal insulin secretion, was also unrelated to epinephrine in amniotic fluid.

Infusion of epinephrine and norepinephrine into the fetal lamb in concentrations comparable to those seen in the fetal circulation during hypoxia may significantly influence the plasma insulin concentration.<sup>28</sup> Thus, after epinephrine infusion the plasma insulin concentration decreased markedly, whereas after norepinephrine infusion insulin levels increased slightly. A positive correlation was present between C-peptide content and norepinephrine content in all three groups studied, although this correlation was not significant in the type I diabetic group. The biologic significance of this relationship is unclear, although it could be speculated that it could represent maturation of these two hormonal systems in parallel.

In conclusion the present data do not suggest that there are any major differences in fetal sympathetic nervous activity between offspring of diabetic women and those of nondiabetic women. This interpretation is also in accordance with recent observations of similar plasma concentrations of epinephrine and norepinephrine at birth in infants of diabetic and nondiabetic mothers.<sup>29</sup>

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# Antenatal phenobarbital for the prevention of neonatal intracerebral hemorrhage

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Forty-six pregnant women less than 35 weeks of gestation were enrolled in a prospective randomized controlled study evaluating the effects of antenatal phenobarbital on neonatal intracerebral hemorrhage. The women were randomly assigned to control ( $n = 22$ ) or treatment ( $n = 24$ ) groups; the treatment group received 500 mg of phenobarbital intravenously. The time interval between the dose of phenobarbital and delivery was  $5.5 \pm 4.8$  hours (mean  $\pm$  SD). The infants in the control group ( $n = 23$ ) and those in the phenobarbital-treated group ( $n = 25$ ) were comparable regarding birth weight, gestational age, and other obstetric and neonatal risk factors associated with intracerebral hemorrhage. The incidence of intracerebral hemorrhage was 56.5% (13 of 23 infants) in the control group and 32% (eight of 25 infants) in the phenobarbital-treated group ( $p = 0.08$ ). Moderate or severe hemorrhage was diagnosed in six of 13 control infants and in none of the phenobarbital-treated infants ( $p < 0.01$ ). The mortality rate was significantly lower in the phenobarbital-treated group (two of 25 infants) than in the control group (eight of 23 infants;  $p < 0.05$ ). Our study suggests that antenatal phenobarbital administration results in a decrease in mortality and in the severity of intracerebral hemorrhage in the preterm neonate. (AM J OBSTET GYNECOL 1986;154:53-7.)

**Key words:** Antenatal, phenobarbital, neonatal intracerebral hemorrhage

The incidence of periventricular, intraventricular, and intracerebral hemorrhage in premature infants with a birth weight  $<1500$  gm or gestational ages less than 35 weeks is 40% to 50%.<sup>1,2</sup> Intracerebral hemorrhage, when it is moderate or severe, is an important cause of neonatal mortality, morbidity, and neurodevelopmental handicap.<sup>1,2</sup> Phenobarbital administered to premature neonates shortly after birth may decrease the incidence and/or the severity of intracerebral hemorrhage.<sup>3,4</sup> The present study was undertaken to evaluate the effect of antenatal phenobarbital administration on the incidence and severity of neonatal intracerebral hemorrhage.

## Patients and methods

The study was performed at the perinatal unit of Hutzel Hospital in Detroit, Michigan. The study population consisted of pregnant women less than 35 weeks of gestation in premature labor and/or with ruptured

membranes. After informed consent was obtained, the women were assigned randomly to a treatment group or a control group by means of a card deck. The women in the treatment group received 500 mg of phenobarbital by slow intravenous infusion during 30 minutes. If delivery did not occur within 24 hours, additional doses of 100 mg of phenobarbital were administered orally every 24 hours until delivery or until labor stopped. Maternal and cord serum phenobarbital concentrations were measured at delivery. The infants' serum phenobarbital concentrations were measured on days 3 and 5. Ultrasound examinations of the infants' heads were performed on days 3 and 14 and the results reviewed by a pediatric radiologist who was not aware of each infant's treatment status. The hemorrhage was classified as mild, moderate, or severe as previously described.<sup>1</sup> Mild hemorrhage was defined as isolated subependymal hemorrhage or subependymal hemorrhage accompanied by a small amount of blood in a normal-sized ventricle. Moderate hemorrhage was defined as intraventricular blood within an enlarged ventricle, and severe hemorrhage was defined as hemorrhage filling the lateral ventricle forming a cast and/or parenchymal extension of hemorrhage. Other clinical data relating to the risk of intracerebral hemorrhage (see below) were recorded prospectively on a data sheet designed for this study.

The study protocol was reviewed and approved by

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**Table I.** Maternal characteristics

	Total		Hemorrhage		No hemorrhage	
	Control infants	Treated infants	Control infants	Treated infants	Control infants	Treated infants
No. of infants	23	25	13	8	10	17
Vaginal delivery	13	14	7	2*	6	12*
Cesarean section	10	11	6	6	4	5
Breech presentation	8	4	7	4	1	0
Complications of pregnancy†	20	21	12	7	8	14

\* $p < 0.05$  (hemorrhage versus no hemorrhage).

†Chorioamnionitis, bleeding, preeclamptic toxemia, or prolonged rupture of membranes.

**Table II.** Neonatal characteristics

	Total		Hemorrhage		No hemorrhage	
	Control infants	Treated infants	Control infants	Treated infants	Control infants	Treated infants
No. of infants	23	25	13	8	10	17
Birth weight (gm)	1380 $\pm$ 595	1377 $\pm$ 531	920 $\pm$ 214	1067 $\pm$ 268	1977 $\pm$ 324	1523 $\pm$ 567*
Gestational age (wk)	30.3 $\pm$ 2.9	30.4 $\pm$ 2.4	28.1 $\pm$ 2.1	29 $\pm$ 1.8	33.2 $\pm$ 1.9	31.1 $\pm$ 2.3*
5 min Apgar score $\leq$ 5	3	1	3	1	0	0
Mechanical ventilation	14	18	12	8	2	10
Air leak	3	1	3	0	0	1
Pressor drugs	6	4	5	2	1	2
Acidosis (pH $<$ 7.20)	7	6	6	1	1	5
Hypercarbia (PCO <sub>2</sub> $>$ 60 mm Hg)	4	4	4	0	0	4
Alkali therapy	7	2	6	0*	1	2
Volume expansion	11	10	9	4	2	6
Fluid therapy (ml/kg/day)						
Day 1	84 $\pm$ 14	82 $\pm$ 5	83 $\pm$ 13	83 $\pm$ 5	87 $\pm$ 15	82 $\pm$ 6
Day 2	97 $\pm$ 27	95 $\pm$ 19	88 $\pm$ 14	89 $\pm$ 8	109 $\pm$ 24	97 $\pm$ 22
Day 3	115 $\pm$ 40	101 $\pm$ 24	98 $\pm$ 12	86 $\pm$ 10*	137 $\pm$ 53	108 $\pm$ 25

\* $p < 0.05$ .

the Human Investigations Committees of Hutzel Hospital and the Children's Hospital of Michigan. Student's  $t$  test (unpaired),  $\chi^2$  analysis, and the Fisher exact test were used for statistical evaluations.

## Results

Sixty-three pregnant women were enrolled in the study; 31 women received phenobarbital and 32 were assigned to the control group. Two women assigned to the phenobarbital-treated group withdrew from the study. An additional 14 women (10 in the control group and four in the phenobarbital-treated group) were also excluded because the premature labor stopped. In all 14 women, the pregnancy continued until term gestation. Two infants, one in the control group (who was the second of a twin gestation) and one in the phenobarbital-treated group, died within 24 hours of birth. Ultrasound examinations of the head were not performed and consents for autopsy were denied. These two infants were also excluded from the study. The

data from 22 women in the control group and 24 women in the phenobarbital-treated group are reported here.

The time interval between enrollment into the study and delivery was comparable in the two groups. All 24 women in the phenobarbital-treated group received 500 mg of phenobarbital and two of these women received additional doses of 100 mg of phenobarbital orally for 2 and 6 days, respectively. There were two twin pregnancies in the control group and one twin pregnancy in the phenobarbital-treated group. The time interval between the last dose of phenobarbital and delivery was  $5.53 \pm 4.8$  hours (mean  $\pm$  SD) and the range was 0.26 to 17 hours. The mean maternal serum phenobarbital level of delivery was  $8.72 \pm 2.01$   $\mu$ g/ml. The mean cord serum phenobarbital level ( $n = 25$ ) was  $8.85 \pm 1.57$   $\mu$ g/ml. The cord:maternal phenobarbital level ratio was  $1.04 \pm 0.21$ . The mean serum phenobarbital concentration in 18 infants on day 3 was  $7.74 \pm 1.72$   $\mu$ g/ml while the phenobarbital con-

centration on day 5 in 17 infants was  $5.62 \pm 1.40 \mu\text{g/ml}$ . No side effects were noted in either mothers or infants in the phenobarbital group.

The infants in the control group ( $n = 23$ ) and in the phenobarbital-treated group ( $n = 25$ ) were comparable regarding the route of delivery, mode of presentation, and complications of pregnancy such as the incidence of chorioamnionitis, bleeding, prolonged rupture of the membranes, and preeclamptic toxemia (Table I). The infants in the control group and the phenobarbital-treated group were similar regarding birth weight, gestational age, number of infants with Apgar scores  $\leq 5$ , the need for ventilatory support, and episodes of acidosis, hypoxemia, and hypercarbia during the first 3 days. The fluid intake during the same period as well as the need for alkali and volume expansion therapy was similar in both groups (Table II).

**Incidence of hemorrhage.** The incidence of intracerebral hemorrhage in the control infants was 56.5% (13 of 23 infants) compared with 32% (eight of 25 infants) in the phenobarbital-treated group ( $p = 0.08$ ; Table III). The diagnosis of hemorrhage was made on the initial ultrasound examination in 10 of 13 infants in the control group and seven of eight infants in the phenobarbital-treated group. Moderate or severe hemorrhage was diagnosed in six of 13 control infants (46%) who had hemorrhage and in none of the phenobarbital-treated infants. The absence of moderate and severe hemorrhage in the phenobarbital-treated infants was significant ( $p < 0.01$ ). The mean birth weight of the six infants in the control group who developed moderate and severe hemorrhage was  $855 \pm 150 \text{ gm}$  while the mean birth weight of the seven infants in the same group who developed mild hemorrhage was  $977 \pm 253 \text{ gm}$  ( $p = 0.15$ ). The mean gestational age of the six infants who developed moderate and severe hemorrhage was  $27.3 \pm 1.2$  weeks, while the mean gestational age of the seven infants with mild hemorrhage was  $28.9 \pm 2.5$  weeks ( $p = 0.13$ ).

The obstetric and neonatal risk factors were compared between the control and phenobarbital treatment groups for the infants who developed hemorrhage ( $n = 21$ ) and those with no hemorrhage ( $n = 27$ ; Tables I and II). Among infants who developed intracerebral hemorrhage, significantly more control infants were delivered vaginally and received more alkali therapy and fluid intake on day 3 (Tables I and II). The characteristics of the 13 infants in the control group who developed hemorrhage and the eight infants in the phenobarbital-treated group who developed hemorrhage are shown in Table IV. The incidence of moderate and severe hemorrhage was not associated with breech delivery among the control group of infants ( $p = 0.1$ ).

**Table III.** Incidence and severity of hemorrhage

	Control infants	Treated infants
No. of infants	23	25
No hemorrhage	10	17
Hemorrhage	13	8
Mild	7	8
Moderate	1	0
Severe	5	0

Mild versus moderate and severe,  $p < 0.01$ .

The mean serum phenobarbital levels were compared between the infants who developed hemorrhage and those who did not. The mean cord serum phenobarbital level in the group with hemorrhage ( $n = 8$ ) was  $9.3 \pm 0.6 \mu\text{g/ml}$  while the level in the group with no hemorrhage ( $n = 17$ ) was  $8.6 \pm 1.8 \text{ ml}$  ( $p = 0.32$ ). There was also no significant difference in the mean maternal serum phenobarbital levels at delivery in the group who developed hemorrhage compared with the group who did not develop hemorrhage ( $9.1 \pm 2.3$  versus  $8.5 \pm 1.9 \mu\text{g/ml}$ ,  $p = 0.52$ ).

**Mortality.** The mortality rate was significantly lower in the phenobarbital-treated group than in the control group (Table V). Severe intracerebral hemorrhage was found to be the cause of death in four of the eight control infants who died. The cause of death in the remaining control group infants was sepsis (two infants), severe hyaline membrane disease (one infant), and severe bronchopulmonary dysplasia at eight months of age (one infant). The two infants in the phenobarbital-treated group who died were found at autopsy to have severe hyaline membrane disease and a ruptured hematoma of the liver, respectively.

### Comment

The incidence of intraventricular and intracerebral hemorrhage in this study was found to be 56.5% in the control group and 32% in the phenobarbital-treated group. This reduction in the incidence of hemorrhage is not as marked a decrease in incidence as was observed in the study by Donn et al.<sup>3</sup> or Ruth et al.<sup>5</sup> Bedard et al.<sup>4</sup> and Morgan et al.<sup>6</sup> did not find any difference in the incidence of hemorrhage in the phenobarbital and control groups. The hemorrhages in the phenobarbital-treated group were significantly less severe than those in the control group in both the present study and that of Bedard et al.<sup>4</sup> We are encouraged by these observations since mortality, long-term morbidity, and neurological outcome correlate with the severity of the hemorrhage.<sup>1,2</sup>

We found a reduction in the mortality due to hemorrhage in the phenobarbital-treated group as compared with the control group in this study. In addition,

**Table IV.** Characteristics of infants who developed hemorrhage

Patient No.	Birth weight (gm)	Gestational age (wk)	Presentation	Delivery	Apgar scores		Air leak	Severity of hemorrhage
					1 min	5 min		
Control group (n = 13)								
1	820	30	Breech	Cesarean section	8	9	—	Mild
2	760	27	Breech	Cesarean section	1	7	—	Severe
3	1000	28	Breech	Cesarean section	5	8	—	Mild
4	710	27	Vertex	Vaginal	4	6	Yes	Severe
5	1230	32	Vertex	Vaginal	2	7	—	Mild
6	580	25	Breech	Vaginal	2	3	—	Mild
7	800	29	Breech	Cesarean section	4	7	—	Mild
8	1000	28	Vertex	Vaginal	4	8	—	Moderate
9	1080	29	Vertex	Vaginal	8	9	—	Severe
10	720	29	Vertex	Vaginal	1	5	Yes	Severe
11	860	26	Breech	Cesarean section	1	8	Yes	Severe
12	1240	30	Vertex	Vaginal	8	9	—	Mild
13	1170	30	Breech/Footling	Cesarean section	1	4	—	Mild
Phenobarbital-treated group (n = 8)								
1	1200	30	Transverse	Cesarean section	1	6	—	Mild
2	650	31	Transverse	Cesarean section	2	9	—	Mild
3	1420	30	Breech	Vaginal	1	5	—	Mild
4	1106	29	Breech	Cesarean section	3	7	—	Mild
5	1090	29	Vertex	Vaginal	3	9	—	Mild
6	740	26	Breech	Cesarean section	2	7	—	Mild
7	960	27	Breech	Cesarean section	7	8	—	Mild
8	1320	31	Vertex	Cesarean section	1	7	—	Mild

**Table V.** Mortality

	Control infants	Treated infants
No. of infants	23	25
Surviving infants	15	23*
Death due to hemorrhage	4	0*

\*p &lt; 0.05 (control versus treated infants).

we found no death related to hemorrhage in the phenobarbital-treated group, although one early death occurred in each study group. A reduction in mortality rate was not observed in the previous studies in which postnatal phenobarbital therapy was used.<sup>3,5</sup> Trolle,<sup>7</sup> in 1968, did demonstrate a decrease in mortality rates for low birth weight infants after antenatal phenobarbital therapy for 3 days. No information is available, however, regarding the incidence of intracerebral hemorrhage in that population.

A major problem with the present study is that, although the infants in the control group were comparable with those in the phenobarbital-treated group, among infants who developed hemorrhage, the control infants required more fluid and alkali therapy in the first 3 days of life than phenobarbital-treated infants. Echoencephalograms were not performed earlier than day 3; hence the relationship between the timing of the hemorrhage and use of alkali or fluid therapy is not documented in this study. However, our previous published experience indicates that hemorrhage occurs in the first few hours of life.<sup>4</sup> We interpret the increased

use of fluid and alkali as a response to the more severe hemorrhage and not as an antecedent.

In the present study, our decision to use a 500 mg dose of phenobarbital was based on the available transplacental pharmacokinetic data. Rapid equilibration of phenobarbital between maternal and fetal serum has been demonstrated.<sup>8</sup> The ratio of cord : maternal serum phenobarbital concentration following antenatal administration has been reported to be 0.95:1.0. Since the volume of distribution of phenobarbital in the adult is approximately 1 L/kg, we attempted to achieve a concentration of 10 µg/ml in the plasma based on a maternal weight of 50 kg. The ratio of cord to maternal serum concentration achieved in this study was  $1.04 \pm 0.21$ .<sup>9</sup> In spite of a wide range of times between the administration of phenobarbital and delivery (0.26 to 17 hours), the mean cord phenobarbital concentration varied little ( $8.85 \pm 1.57$  µg/ml). The protective effect of phenobarbital was noted in this study at serum levels that are considerably lower than the anticonvulsant levels observed in the previous studies with the use of phenobarbital therapy in the neonate.<sup>3,4</sup>

Phenobarbital has been administered during pregnancy for specific indications. It has been recommended for use throughout the length of the pregnancy in the management of the pregnant epileptic woman.<sup>8</sup> Phenobarbital has also been suggested for use in pregnant women in premature labor at less than 37 weeks' gestation, along with betamethasone and ritodrine, to prevent respiratory distress syndrome in the neonate.<sup>10</sup> Finally, phenobarbital has been given ante-



nately for varying lengths of time (3 days to 8 weeks) for the prevention of neonatal hyperbilirubinemia.<sup>11</sup> The side effects of antenatal phenobarbital usage such as withdrawal symptoms, alterations in clotting mechanisms, and hypocalcemia have been observed only after prolonged usage throughout pregnancy.<sup>8</sup> No long-term effects have been observed in children at 5 years of age born to mothers treated with phenobarbital antenatally to prevent hyperbilirubinemia.<sup>12</sup>

The use of postnatal phenobarbital therapy to protect against neonatal intracerebral hemorrhage has met with conflicting results. Donn et al.,<sup>3</sup> in 1981, demonstrated that intravenous phenobarbital given in doses sufficient to achieve anticonvulsant serum levels within 12 to 18 hours of birth resulted in a 70% decrease in the incidence of hemorrhage. Bedard et al.,<sup>4</sup> using a similar dosage schedule, have demonstrated a decrease in the severity of hemorrhage, although the control group infants who developed hemorrhage in that study weighed less than the phenobarbital-treated group. Morgan et al.,<sup>6</sup> using a different dosage regimen (a 20 mg/kg dose within a few hours after birth), did not find any change in the incidence or severity of hemorrhage in the phenobarbital-treated group. Whitelaw et al.<sup>13</sup> performed a double-blind randomized trial of phenobarbital (20 mg/kg dose) versus placebo and found no decrease in the incidence of hemorrhage. There were, however, fewer parenchymal hemorrhages in the treatment group. Ruth et al.,<sup>5</sup> evaluating the effect of phenobarbital on intraventricular hemorrhage and late neurological handicap, found a decrease in incidence of hemorrhage in the phenobarbital-treated group as well as a possible beneficial effect at follow-up.

The majority of neonatal periventricular and intraventricular hemorrhages occur within the first 24 hours of life.<sup>4</sup> Neonatal intracerebral hemorrhage has been associated with both perinatal and neonatal risk factors. In a previous study performed by us on an outborn population, half the otherwise eligible infants were excluded because they had evidence of hemorrhage on admission to the neonatal unit.<sup>4</sup> It is possible that infants with a potential for early cerebral bleeding may benefit from antenatal phenobarbital because it acts during the perinatal events associated with risk of hemorrhage.

The neuroprotective effect of antenatally administered phenobarbital in the animal experimental model has been well documented.<sup>14</sup> Phenobarbital has been found to be beneficial in the treatment of neonates and children with birth asphyxia, seizures, and meningitis.<sup>15</sup> The mode of action of phenobarbital in the prevention of intracerebral hemorrhage in newborn infants is not well understood. One possible mechanism is that phenobarbital abolishes the hypertensive peaks that occur during spontaneous activity and nursing procedures,

which may contribute to the development of intracerebral hemorrhage.<sup>16</sup>

In conclusion, we have performed a prospective controlled randomized study with the use of antenatal phenobarbital therapy and have demonstrated a significant decrease in the severity of hemorrhage and a significant decrease in mortality rate in the phenobarbital-treated group as compared with the control group. We suggest that further trials be carried out before antenatal phenobarbital be recommended for routine use.

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# The role of biofeedback in Kegel exercise training for stress urinary incontinence

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This study examined the effectiveness of teaching pelvic floor exercises with use of bladder-sphincter biofeedback compared to training with verbal feedback based on vaginal palpation in 24 women with stress urinary incontinence. Verbal feedback training consisted of instructing the patient to squeeze the vaginal muscles around the examiner's fingers and providing her with verbal performance feedback. Biofeedback patients received visual feedback of bladder pressure, abdominal (rectal) pressure, and external anal sphincter activity. The biofeedback group improved the strength and selective control of pelvic floor muscles; the verbal feedback group did not. Both groups significantly reduced the frequency of incontinence. The biofeedback group averaged 75.9% reduction in incontinence, significantly greater than the 51.0% reduction shown by the verbal feedback group. Twelve of 13 patients in the biofeedback group improved by 60% or better. Six patients in the verbal feedback group improved by 68% or better, and five were less than 30% improved. (AM J OBSTET GYNECOL 1986;154:58-64.)

**Key words:** Biofeedback, Kegel exercises, stress incontinence, physiotherapy

Stress urinary incontinence is the involuntary loss of urine that occurs following a sudden rise in intra-abdominal pressure brought on by physical activities such as coughing, sneezing, jogging, or lifting. Incontinence results when a corresponding rise in bladder pressure exceeds urethral resistance. One commonly accepted etiologic factor is perinatal damage to the supporting tissues of the pelvic floor. The precise mechanism of urine loss is a topic of debate. Anatomic explanations emphasize the loss of the urethrovesical angle because of overstretched or damaged pelvic floor tissues. Functional explanations point to a lack of awareness of voluntary control over pelvic floor muscles or a failure of the striated muscle of the distal urethral sphincter to contract during transient rises in intra-abdominal pressure. According to either analysis, urethral resistance is inadequate in the stress incontinent woman.

One method of improving urethral resistance and urinary control is the active exercise of pelvic floor muscles. As early as 1948 A. H. Kegel advocated this approach and reported positive results with young as well as elderly stress incontinent women.<sup>1,2</sup> Kegel developed what is now regarded as a biofeedback device, the perineometer, to be used by patients learning vaginal mus-

cle exercises. It consisted of a vaginal chamber attached to a manometer which measured perineal muscle contractions and provided visual feedback to patient and physician. The first step in muscle reeducation was to establish awareness of function of the muscles. Then regular exercise was encouraged to improve muscle coordination and strength.

Kegel exercises have continued to be described and advocated but typically without the benefit of the biofeedback apparatus. Under these conditions the exercises have apparently been less effective than originally described by Kegel. They are usually recommended only for women with mild incontinence or as an adjunct to surgical intervention. Some gynecologic texts make no mention of physiotherapy as a treatment for incontinence.

The premise for Kegel's procedure was that stress incontinent women need first to gain awareness of the function of the pubococcygeal muscle. The biofeedback device served this vital purpose. Unfortunately, when exercises are merely described, women do not always understand the exercise. The conscientious care provider who takes the time to teach the patient by asking her to squeeze the vaginal muscles around the examiner's finger and then providing verbal performance feedback is one step closer to assuring accurate practice. However, the direct effects of this training procedure on muscle activity have yet to be evaluated, and it is not known with what degree of accuracy pelvic floor contractions can be detected via palpation. Without physiologic measures there is no certainty that the correct response is being taught. In addition there remains the issue of response selectivity. The correct response

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may be accompanied by irrelevant responses such as contractions of gluteal muscles. Of more concern is the counterproductive tendency of women to tense abdominal wall muscles, which increases bladder pressure and therefore enhances the probability of incontinence.

It is the hypothesis of the present study that while many women can benefit from Kegel exercises taught in the usual fashion, physiologic feedback of performance (biofeedback) is an important component in teaching pelvic floor exercises because it provides for discriminability of muscle contraction, in the absence of which learning cannot be expected to occur. Acquisition of a reliable, selective response cannot occur without information about the outcome of attempts. Through physiologic feedback, otherwise undetected or poorly detected responses are amplified so that the patient can observe and learn from the results of her efforts.

## Methods

**Screening.** Twenty-seven women between the ages of 29 and 64 underwent urologic evaluation. All complained of involuntary loss of urine associated with coughing, sneezing, lifting, or other physical activity.

Urologic evaluation of these women included medical history, urinalysis, urodynamic testing, and stress testing for stress incontinence. The purpose of the examination was to differentiate patients with stress incontinence from those with other disorders. Patients with detrusor hyperreflexia (uninhibited detrusor contractions at a volume of <350 ml) were excluded and those with urinary tract infection were deferred until treated medically.

**Baseline.** During a 4-week baseline period patients were instructed to void every 2 hours during the waking day and to keep detailed symptom diaries to document voiding patterns and frequency of incontinence. Patients noted the size of each urinary accident as leakage or large volume loss and recorded the events or circumstances associated with each accident. Patients were then assigned to one of the two training groups so as to balance the groups on age and frequency of incontinence during baseline.

## Assessment

**Stress test.** In the first and last training sessions the adequacy of the bladder outlet was assessed with the following procedure: A No. 14 French catheter was passed into the bladder. After residual urine was removed, 350 ml of sterile water were infused. The patient was then engaged in three provocative maneuvers: coughing in the lithotomy position, coughing in the standing position, and squatting. Loss of urine during any of these activities was recorded.

**Sphincter strength.** With the patient in the lithotomy position, a rectal tube with three small balloons was

inserted.<sup>3</sup> One balloon was positioned inside the rectum and used to measure intra-abdominal pressure. Another balloon, which was positioned at the anal opening, measured activity of the external anal sphincter. Provided there is no damage to the sphincters, external anal sphincter activity reflects activity in the external urethral sphincter, since both are innervated by the pudendal nerve.<sup>4,5</sup> A third balloon, positioned at the internal anal sphincter, stabilized the recording apparatus but provided no data.

On three trials, each patient was asked to imagine that she was attempting to hold back the flow of urine and to squeeze the muscles as tightly as possible for 10 seconds on each attempt. Bladder pressure, abdominal (rectal) pressure, and external anal sphincter activity were recorded on a polygraph during these trials, but no feedback was provided. This assessment was also repeated in the last treatment session.

**Gynecologic evaluation.** Patients were examined by a gynecologist (C. R.) who was blind to the patient's treatment group. The purpose of the examination was to identify criteria that might be expected to influence subsequent outcome of physiotherapy. The gynecologist interviewed each patient regarding the number of vaginal deliveries and history of gynecologic surgery. Then a pelvic examination was performed to assess (1) vesicourethral angle, (2) cystocele, (3) cervical prolapse, and (4) rectocele. Ordinarily, the gynecologic evaluation was performed in the first treatment session but in some cases was postponed until the second or third treatment.

**Training.** Training in each group included four bi-weekly training sessions approximately 1 hour in duration.

**Bladder-sphincter biofeedback.** During biofeedback training the polygraph tracings described above were visible to the patient, thus providing her with immediate and simultaneous visual feedback of bladder, sphincter, and abdominal pressures as she learned sphincter exercises. In addition to visual feedback, verbal instructions and verbal reinforcement of appropriate muscle responses were used to teach contraction of the sphincter muscles while minimizing intra-abdominal pressure. In each session patients practiced 25 exercises, including the three assessment trials. Each exercise consisted of a 10-second contraction followed by 10 seconds of relaxation.

The purpose of this sphincter training was to teach skills that could then be used to prevent incontinence under the usual circumstances of daily life. Therefore generalization was addressed in two ways. First, when patients demonstrated adequate sphincter control, they practiced performing on alternate trials with and without feedback to improve performance when the polygraph was unavailable. Second, patients were trained



**Table I.** Characteristics of the training groups before intervention

	Biofeedback (n = 13)	Verbal feedback (n = 11)	t	p
Age (yr)	47.9 ± 12.1	40.7 ± 10.9	1.51	NS
Frequency of incontinence (accidents, No./wk)	6.9 ± 4.6	5.8 ± 4.5	0.54	NS
Duration of incontinence (yr)	10.8 ± 13.3	9.6 ± 5.8	0.25	NS
No. of vaginal deliveries	2.1 ± 1.8	2.1 ± 1.6	0.02	NS
No. of women with				
Previous surgical repair for incontinence (%)*	2 (15.4)	2 (18.2)		NS
Previous physiotherapy (%)*	1 (7.7)	1 (9.1)		NS
Occasional urge incontinence (%)*	6 (46.2)	5 (45.4)		NS
Positive stress test (%)*	6 (46.2)	4 (36.4)		NS

\*Group comparisons were made with use of Fisher's exact probability test.

to actively use these muscles during physical stress. In the clinic they practiced contracting the muscles immediately before and during voluntary coughing.

**Verbal feedback.** During exercise training without biofeedback the bladder catheter and rectal tube were removed. The training consisted of insertion of two gloved fingers into the vagina and instruction to the patient to squeeze the vaginal muscles around them. The therapist's hand was placed lightly on the lower abdomen to detect tensing of abdominal muscles. Verbal instruction, verbal feedback of vaginal muscle contraction, and verbal reinforcement of appropriate responses were used to teach selective contraction of vaginal muscles while relaxing abdominal muscles. As in the biofeedback training, each session consisted of 25 exercises and patients practiced the active use of their muscles during physical stress.

**Home program.** Patients in both groups were given verbal and written instructions for home practice which included 51 pelvic floor exercises divided into three sessions of 17 exercises each. Patients were advised to practice in various positions including lying down, sitting, and standing and, when possible, to integrate the exercises into other daily activities such as standing in line, sitting in a car at a stoplight, washing dishes, or sitting at a desk. They were encouraged to attend closely to those activities and events that previously had resulted in incontinence and to actively squeeze the pelvic floor muscles just before and during these activities. Finally, to assist in strengthening the proper muscles, patients were instructed to practice interruption or slowing of the urinary stream during voiding. Patients were instructed to continue voiding every 2 hours and to keep symptom diaries at home so that progress could be monitored.

**Follow-up.** Six months following the final training session, patients returned to the clinic for follow-up evaluation. Patients kept symptom diaries for the 2 weeks before and 2 weeks following the clinic visit.

## Results

Urologic evaluation led to exclusion of three patients who exhibited detrusor hyperreflexia during urody-

namic testing. None of the women exhibited stress-induced hyperreflexia. The 24 remaining women were trained. Thirteen women were assigned to biofeedback training and 11 to verbal feedback training without biofeedback.

**Group characteristics.** Table I describes the characteristics of the two groups before training. As planned, the groups did not differ significantly in age or in frequency of incontinence during the baseline phase. These women had histories of stress incontinence ranging in duration from 10 weeks to 45 years (mean, 10.3 years) without group differences. Four patients (two in each group) reported previous surgical repair for stress incontinence (e.g., Marshall-Marchetti-Krantz procedure) which resulted in temporary relief (0-10 months). Two patients (one in each group) had previously failed to improve with Kegel exercises. Nearly half of the patients in each group reported a secondary problem with urge incontinence.

**Sphincter strength.** To assess the strength of the external anal sphincter, the polygraph tracings of the three assessment trials before and after treatment were measured and scored on four dimensions. Each contraction was scored for (1) peak amplitude and for (2) duration (time in seconds that the contraction was sustained above 5 mm Hg). The maximum scored duration was ten seconds because patients were at that time instructed to relax. In addition, the amplitude of changes in (3) bladder and (4) rectal (intra-abdominal) pressures which accompanied sphincter contractions were recorded.

Before training the two groups did not differ significantly on amplitude of sphincter contractions ( $t = 0.45$ ) or duration ( $t = 0.29$ ) of contractions. Similarly, no differences were found in the magnitude of change in bladder pressure ( $t = 0.33$ ) or rectal pressure ( $t = 0.34$ ), indicating that the groups were similar in the degree to which they tensed abdominal muscles and thus increased bladder pressure during sphincter contractions.

Following training the biofeedback group demonstrated improved ability to sustain sphincter contractions ( $t = 2.64$ ,  $p < 0.05$ ) and to minimize bladder

**Table II.** Results of training on frequency of incontinence

Biofeedback (n = 13)				Verbal feedback (n = 11)			
Age	Accidents (No./wk)		% of improvement	Age	Accidents (No./wk)		% of improvement
	Baseline	After training			Baseline	After training	
60	0.5	0.0	100	29	0.7	0.5	29
32	0.5	0.3	40	42	0.8	0.0	100
54	2.3	0.3	87	47	1.7	2.0	-18
39	3.2	0.5	84	30	2.0	0.5	75
41	5.6	0.0	100	58	4.7	4.3	8
32	6.1	2.0	67	34	6.0	5.0	17
64	6.3	1.3	79	33	6.1	2.0	67
34	7.3	1.5	79	31	6.7	1.4	79
59	8.3	2.7	67	37	10.3	0.3	97
42	10.0	0.3	97	48	11.8	8.5	28
46	11.3	4.4	61	59	13.5	2.8	79
56	11.8	4.1	65				
64	16.0	6.2	61				
Mean	47.9	6.9	75.9	40.7	5.8	2.5	51.0
SD	12.1	4.6	17.9	10.9	4.5	2.6	39.6

**Table III.** Pretraining characteristics of successful and unsuccessful control patients\*

	Successful* (n = 5)	Unsuccessful (n = 5)	t	p
Peak amplitude of sphincter contraction (mm Hg)	20.6 ± 5.6	9.6 ± 9.5	2.22	<0.05
Duration of sphincter contraction (sec)	9.1 ± 1.8	2.9 ± 4.2	3.02	<0.05
Peak amplitude of increase in bladder pressure (mm Hg)	3.6 ± 1.0	8.4 ± 6.1	1.71	NS
Peak amplitude of increase in rectal pressure (mm Hg)	4.8 ± 1.8	5.1 ± 4.6	0.11	NS

\*Data presented are from five of the six successful patients. One patient did not tolerate the procedure for assessing sphincter strength.

pressure during these contractions ( $t = 2.23$ ,  $p < 0.05$ ). The verbal feedback group showed no improvement in any of the four dimensions.

**Effects of training on incontinence.** With use of the patients' home records the weekly frequency of incontinence was calculated for the baseline phase and the 4 weeks immediately following treatment. Both groups demonstrated significant reductions in the frequency of incontinence. As shown in Table II, the biofeedback group reduced incontinence from an average 6.9 accidents per week to 1.8 accidents per week in the post-treatment period ( $t = 5.85$ ,  $p < 0.001$ ). In the verbal feedback group, incontinence decreased from 5.8 accidents per week to 2.5 per week ( $t = 2.88$ ,  $p < 0.01$ ).

The biofeedback group demonstrated an average 75.9% reduction of incontinence, significantly greater than the 51.0% reduction shown by the verbal feedback group ( $t = 2.04$ ,  $p < 0.05$ ). With one exception, all patients in the biofeedback group improved by 60% or better. On the other hand, the verbal feedback group displayed more variability. Six patients improved by 68% or better, four were <30% improved, and one regressed. Thus while all biofeedback patients made

significant gains, nearly half of the verbal feedback group failed to respond.

For the purpose of further evaluation, the five patients who reduced incontinence <30% in the verbal feedback treatment were compared to five of six who improved by 68% or better. Sphincter strength of one patient was not assessed because she did not tolerate the procedure. Unsuccessful patients were found to be significantly different from successful patients in two dimensions. Their sphincter contractions were of lower peak amplitude and shorter duration (Table III) before treatment as well as following treatment. Biofeedback patients with comparable sphincter contractions before treatment subsequently improved by 40% or better.

**Stress test.** Before treatment, six biofeedback patients and four verbal feedback patients exhibited loss of water with stress testing (Table I). Following treatment, only two biofeedback and one verbal feedback patient were unable to prevent such leakage. Thus both groups improved on this dimension. Results of the stress test did not appear to be related to treatment outcome. In the control group, 40% (two of five) of

**Table IV.** Results of pelvic examination

	Biofeedback (n = 11)		Verbal feedback (n = 10)	
	n	%	n	%
Suburethral angle				
Good	6	54	2	20
Fair	4	36	4	40
Poor	1	9	4	40
Cystocele				
None	4	36	1	10
Moderate	7	64	9	90
Severe	0	0	0	0
Cervical prolapse				
Absent	3	27	2	20
First-degree	5	45	6	60
Second-degree	3	27	2	20
Third-degree	0	0	0	0
Rectocele				
Absent	0	0	0	0
First-degree	3	27	2	20
Second-degree	6	54	8	80
Third-degree	2	18	0	0

unsuccessful patients and 33% (two of six) of successful patients had a positive stress test.

**Gynecologic evaluation.** Eleven biofeedback patients and 10 verbal feedback patients were evaluated gynecologically. Table IV displays the results of these evaluations. The majority of each group displayed moderate cystocele and rectocele and first-degree cervical prolapse. The two groups were compared with use of Fisher's exact probability test, which requires dichotomous classification. For the purposes of this analysis, patients were classified according to the presence or absence of cystocele, cervical prolapse, rectocele, and a good or fair suburethral angle compared to a poor angle. There were no significant differences between the treatment groups.

The only noteworthy difference between the groups was the large proportion of biofeedback patients who presented with "good" vesicourethral angles. Only one biofeedback patient as opposed to four verbal feedback patients was found to have a poor suburethral angle. Further analysis explored the possibility that the greater proportion of patients with poor urethral support had accounted for the poorer performance of the verbal feedback group as a whole. The five patients who demonstrated successful response to verbal feedback training were compared to the treatment failures. Two important facts emerged. First, three of the four patients with poor suburethral angles were treated successfully. In other words, only one of the five failures had a poor suburethral angle. Second, three of the four measures of urethral support showed the failures to be similar to or slightly better than the successful patients. It was clear that the treatment failures were at least equivalent to the successful patients on these measures of pelvic support. Therefore lack of urethral support

could not have accounted for treatment failures, and assessment of anatomic features via pelvic examination provides a poor prediction of response to physiotherapy.

**Six-month follow-up.** Ten of 13 biofeedback patients (77%) and six of 11 verbal feedback patients (55%) consented to participate in the follow-up phase of the study. The biofeedback patients who returned showed an insignificant decline from a mean 75.0% reduction of incontinence immediately following training to a mean 68.0% reduction at follow-up ( $t = 0.58$ ,  $p > 0.05$ ). The six verbal feedback patients also demonstrated insignificant change from a mean improvement of 45.7% immediately following training to 56.7% reduction of incontinence at follow-up ( $t = 1.09$ ,  $p > 0.05$ ). Overall, the training effects appear to be maintained.

Six months after training the five verbal feedback patients who were <30% improved were offered biofeedback training, and all declined. Therefore the effects of a crossover could not be evaluated.

### Comment

These findings indicate that biofeedback is more effective than verbal feedback based on vaginal palpation for teaching selective sphincter control. Only the biofeedback group demonstrated increased sphincter strength with training and improved ability to minimize intra-abdominal pressure. The verbal feedback group demonstrated no significant physiologic changes. Furthermore, training resulted in significant reduction of incontinence in 92% of patients who received biofeedback and 55% of patients who did not. Thus bladder-sphincter biofeedback appears to greatly increase the likelihood of successful treatment, particularly for patients with weak sphincter muscles.

The verbal feedback procedure used in this study was an elaborate intervention compared to the usual methods for teaching Kegel exercises. Patients were seen in four treatment sessions and practiced 100 exercises with the therapist. Even under these conditions, training was less effective than when biofeedback was used.

The evidence is that merely instructing patients to exercise or even teaching them to contract the vaginal muscles around the examiner's finger will not produce the maximum possible benefit. Many women will fail with these methods who could probably achieve significant improvement with biofeedback. In fact, some of the patients in this study and in a previous study<sup>3</sup> had failed with prior physiotherapy treatment and subsequently improved with biofeedback. Therefore biofeedback appears to maximize the benefits of physiotherapy and should be considered before surgical intervention is contemplated.

These findings should not be unexpected. The phys-



ologic feedback used in this study differs from the therapist's feedback on four dimensions that would predict its superiority.

First, the information provided by verbal feedback was basically binary in nature whereas biofeedback yielded information that was clearly better graded. With verbal feedback the therapist informed the patient whether contractions were weak or strong relative to her prior attempts but without precise information about the degree of strength or weakness. Physiologic feedback gave precise quantitative information, allowing the patient to compare the strength of her current contraction with relaxation or with previous contractions. Research on motor learning has shown that quantitative feedback is a significant performance variable in the acquisition of motor responses such as line drawing<sup>6</sup> and reaction time.<sup>7</sup> In line-drawing tasks, telling subjects whether their performance was right or wrong was less effective than reporting the degree of error in  $\frac{1}{8}$  inch units.<sup>8</sup> Informing subjects of their exact reaction time was more effective for learning a particular reaction time than simply telling them the response was too fast or too slow.<sup>7</sup> Similarly, proportional (quantitative) feedback has proved more useful than binary feedback in acquisition of autonomic function such as altering heart rate<sup>8</sup> and in modification of frontalis muscle activity.<sup>9</sup>

The second dimension on which the verbal and physiologic feedback differed was frequency of information feedback. Physiologic feedback was continuous, providing moment-to-moment performance information. Verbal feedback was necessarily intermittent. The therapist provided several bits of information per trial, but it was pooled information and the first bit was given only after active contraction had been effected. An analogous situation exists in heart rate conditioning experiments in which feedback given after every beat results in more rapid acquisition than feedback after every 5 or 10 beats.<sup>10</sup>

Inherent in the dimension of feedback frequency is the factor of immediacy. In physiologic feedback in which information is continuous it is also immediate. Intermittent verbal feedback involves a delay. Animal research has demonstrated that performance is adversely affected when reinforcement (feedback) is delayed.<sup>11-16</sup> Delay of feedback can also disrupt certain types of human performance, a classic example being the manifestation of serious speech disturbance in normal subjects given speech feedback delayed by only 200 msec.<sup>17, 18</sup>

Finally, physiologic feedback and verbal feedback may have differed on the dimension of accuracy, which has been shown to be essential to learning. Nonveridical or irrelevant feedback has been used as a control procedure in a number of studies and has proved to disrupt acquisition<sup>19, 20</sup> and in some cases results in poorer per-

formance than no feedback.<sup>6</sup> The accuracy of verbal feedback may be reduced by the inherent delay that makes it possible for positive feedback to be presented to the patient at the moment an incorrect response occurs. Verbal feedback may also be inaccurate because it is based on clinical judgment derived from palpation.

Within the verbal feedback group of this study, successful patients were found to have stronger sphincter muscles than unsuccessful patients both before and after treatment. It is possible that strong muscle contractions were more discriminable, providing the successful patients with proprioceptive feedback that guided their performance and skill acquisition. The weaker muscles of unsuccessful verbal feedback patients may have been less discriminable, thus depriving them of such feedback. When patients with comparable sphincter weakness were treated with biofeedback, results were good, most likely because biofeedback compensated for the deficit in proprioception. These findings are consistent with early feedback research, which demonstrated that subjects who received electromyographic feedback showed more rapid acquisition of an invisible muscle contraction (thumb twitch) than did subjects who received no feedback.<sup>21</sup> Thus it has long been known that physiologic feedback facilitates acquisition of responses that are otherwise undetected.

The fact that approximately half of verbal feedback patients were treated successfully raises the issue of patient selection. Ideally one would want to predict which patients will benefit significantly from instructions or verbal feedback and which ones will do better with biofeedback. Unfortunately, this study reveals no clear-cut predictors. None of the anatomic features assessed by the pelvic examination predicted response to physiotherapy. A possible exception is the unexpected finding that failures of the verbal feedback procedure had better suburethral angles than did their successful counterparts. Failures did have significantly weaker sphincter muscles as measured by the polygraph. However, if patients are so evaluated with the polygraph equipment, the most reasonable course of action is to proceed with biofeedback with use of the polygraph tracing.

Another seemingly rational approach would be to proceed with verbal feedback training and switch to biofeedback training if results are inadequate. Our experience cautions against this. Each of the verbal feedback failures was subsequently offered biofeedback training, and each refused. Their efforts had ended in failure, and they expressed discouragement with physiotherapy. For this additional reason, if polygraph equipment is available, biofeedback training should be provided for all appropriate physiotherapy patients.

The data show clearly that for many, if not most women, biofeedback can be a cost-effective method for teaching pelvic floor exercises and reducing stress in-

continence. It can be applied by a nurse or other health professional in an outpatient setting, and the cost of equipment is nominal. Considering that it is less expensive than surgical intervention and involves significantly lower risk, it might be considered the first treatment offered to stress incontinent women.

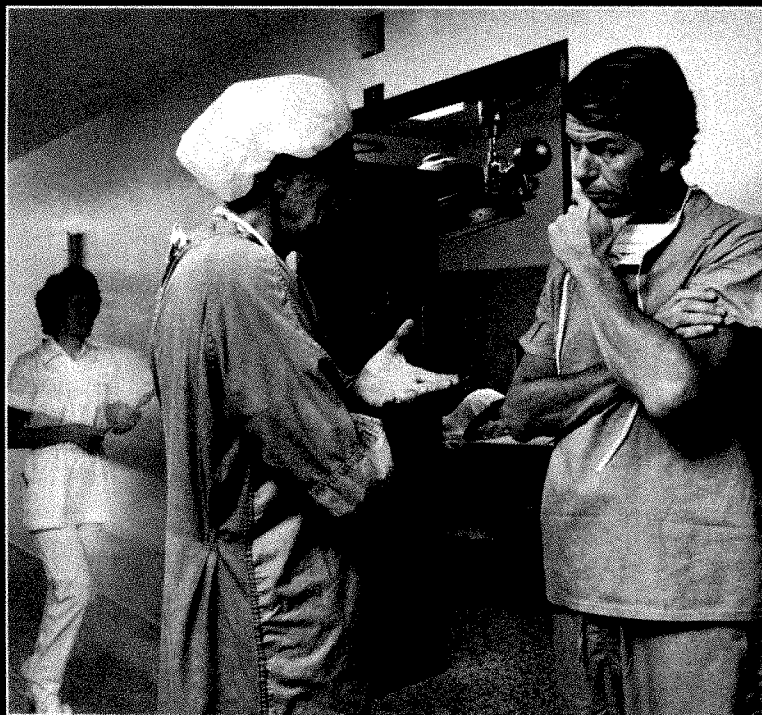
Urologic evaluation of patients was provided by Charles B. Brendler, M.D., Department of Surgery (Urology), Francis Scott Key Medical Center. Ruth Ann Arty, B.S.W., assisted in the clinical management of patients.

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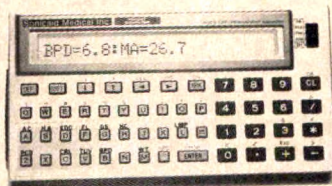


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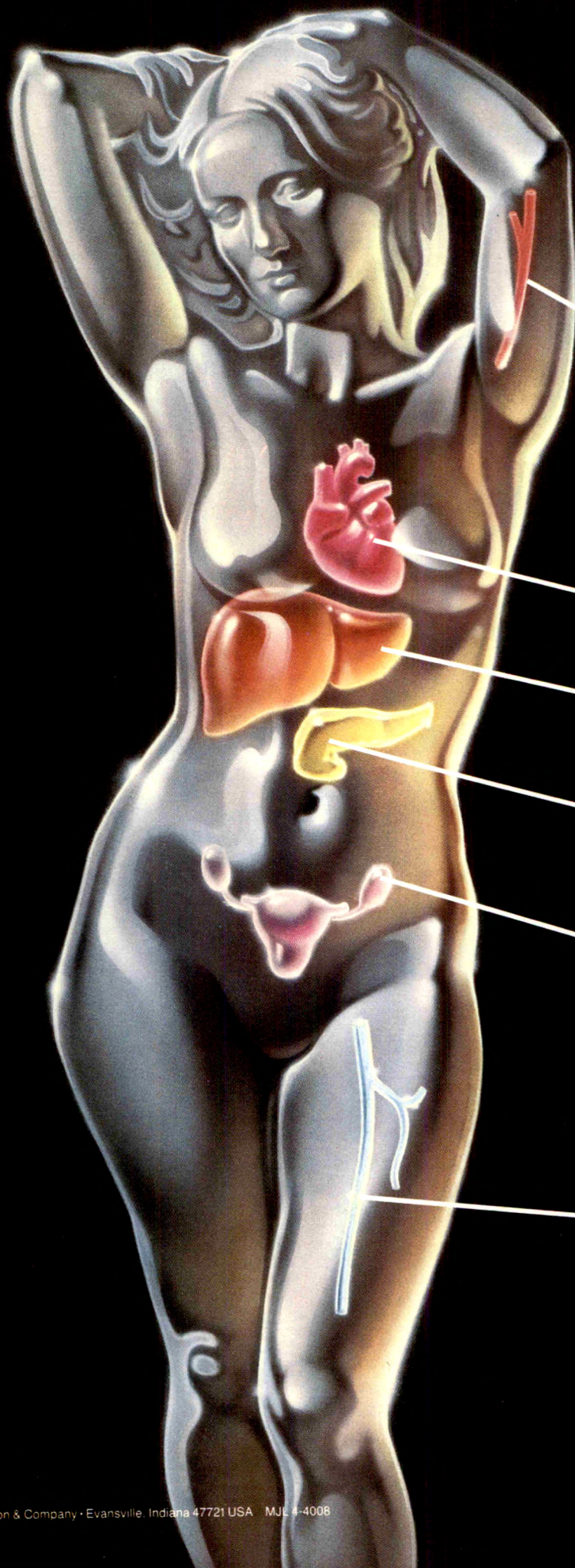


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#As noted in the OVCON labeling, a tendency toward increased blood coagulability has been observed in a significant percentage of patients on oral contraceptives. The findings in this present study represent preliminary information and are yet to be confirmed. Prolonged follow-up will be necessary to see if clinical differences in coagulation states can be observed in patients receiving OVCON-35.

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**Dose-related Risk of Thromboembolism from Oral Contraceptives:** Two studies have shown a positive association between the dose of estrogens in oral contraceptives and the risk of thromboembolism. For this reason, it is prudent and in keeping with good principles of therapeutics to minimize exposure to estrogen. The oral contraceptive product prescribed for any given patient should be that product which contains the least amount of estrogen that is compatible with an acceptable pregnancy rate and patient acceptance. It is recommended that new acceptors of oral contraceptives be started on preparations containing 50 mcg, or less of estrogen.

**Contraindications:** Oral contraceptives should not be used in women with any of the following conditions: 1) Thrombophlebitis or thromboembolic disorders. 2) A past history of deep vein thrombophlebitis or thromboembolic disorders. 3) Cerebral vascular or coronary artery disease. 4) Known or suspected carcinoma of the breast. 5) Known or suspected estrogen dependent neoplasia. 6) Undiagnosed abnormal genital bleeding. 7) Known or suspected pregnancy. 8) Benign or malignant liver tumor which developed during the use of oral contraceptives or other estrogen-containing products.

#### Warnings:

**Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. Women who use oral contraceptives should be strongly advised not to smoke. Use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.**

1) **Thromboembolic Disorders and Other Vascular Problems.** An increased risk of thromboembolic and thrombotic disease associated with use of oral contraceptives is well established. Three principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of oral contraceptives are 4 to 11 times more likely than nonusers to develop these diseases without evident cause. In a study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater. An increased risk of myocardial infarction associated with the use of oral contraceptives has been reported. Studies conducted in the United Kingdom found that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preclampsia/tokemia) the higher the risk of developing myocardial infarction, regardless of whether the patient was an oral contraceptive user or not. Oral contraceptives, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that oral contraceptive users who do not smoke (smoking considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction compared to nonusers who do not smoke. Oral contraceptive users who smoke have about a 5-fold increased risk of fatal infarction compared to users who do not smoke but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. The amount of smoking is a very important factor. British investigators concluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in oral contraceptives; preparations containing 100 mcg, or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. The relative risk of thromboembolic disease associated with progestin-only oral contraceptives has not been determined. Cases of thromboembolic disease have been reported in women using progestin-only products, and they should not be presumed to be risk-free. The overall excess mortality rate annually from circulatory diseases for oral contraceptive users was estimated to be 20 per 100,000 (ages 15-34, 5/100,000; ages 35-44, 33/100,000; ages 45-49, 140/100,000), the risk being concentrated in older women, in those with a long duration of use, and in cigarette smokers. The highest risk was found in heavy cigarette smokers (15 or more cigarettes per day) who used oral contraceptives and were aged 40 or older. Women who smoke should be advised not to use oral contraceptives. The use of oral contraceptives in women over age 40 with other risk factors is not recommended. The mortality associated with all of the methods of birth control is low compared to the risk of childbirth, with the exception of oral contraceptive users who smoke and are over age 40. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with oral contraceptives increases with age after approximately age 30 and for myocardial infarction is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of preclampsia/tokemia, and especially by cigarette smoking. The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. If feasible, oral contraceptives should be discontinued at least 4 weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization. 2) **Ocular Lesions.** There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of oral contraceptives. Discontinue the medication if there is unexplained sudden or gradual, partial or complete loss of vision; sudden onset of proptosis or diplopia; papilledema; or retinal vascular lesions, and institute appropriate diagnostic and therapeutic measures. 3) **Carcinoma.** Long term administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina and liver. In humans, one study investigated the first 21 cases of endometrial adenocarcinoma in women on oral contraceptives reported to a registry. Of those women without predisposing risk factors for this disease, nearly all occurred in women who had used a sequential oral contraceptive. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestin-only oral contraceptives. No increase in breast cancer in women taking oral contraceptives has been reported although one study reported an increased risk of breast cancer in subgroups of women treated using oral contraceptives with documented benign breast disease. There is at present no confirmed evidence from human studies of an increased risk of cancer associated with oral contraceptives. Close clinical surveillance of all women taking oral contraceptives is essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use oral contraceptives. 4) **Hepatic Adenoma.** Benign hepatic adenomas have been found to be associated with the use of oral contraceptives. One study reported a higher risk associated with oral contraceptive formulations with high hormonal potency. Although benign and rare, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short term as well as long term users of oral contraceptives. Two studies relate the risk with duration of contraceptive use, the risk being much greater after 4 or more years of oral contraceptive use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass, or shock. A few cases of hepatocellular carcinoma have been reported in women taking oral contraceptives. The relationship of these drugs to this type of malignancy is not known at this time. 5) **Use in Pregnancy, Birth Defects in Offspring and Malnutrition in Female Offspring.** Fetal abnormalities have been reported to occur in the offspring of women who have taken progestogens and/or estrogens during pregnancy. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. A high percentage of such exposed women (30% to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Similar data are not available with the use of other estrogens but it cannot be presumed that they would not induce similar changes. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 in 1000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications and

there is no evidence from well-controlled studies that progestins are effective for these uses. Increases in chromosomal aberrations have been reported in women who become pregnant soon after ceasing oral contraceptive therapy. Embryos with these anomalies are virtually always spontaneously aborted. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping oral contraceptives is unknown. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period and further use of oral contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is confirmed the patient should be apprised of the potential risks to the fetus and the advisability of pregnancy continuation should be discussed in light of these risks. It is also recommended that women who discontinue oral contraceptives with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend three months. Administration of progestin-only or progestin-estrogen combinations to induce withdrawal bleeding should not be used as a test for pregnancy. 6) **Gall Bladder Disease.** Studies have reported an increased risk of surgically confirmed gallbladder disease appearing after one year of oral contraceptive use and doubling of the risk after 4 or 5 years of use. 7) **Carbohydrate and Lipid Metabolic Effects.** A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. For this reason, prediabetic and diabetic patients should be carefully observed while receiving oral contraceptives. Increased serum levels of triglycerides and total phospholipids have been observed in oral contraceptive users. The clinical significance of this observation is unknown at this time. 8) **Elevated Blood Pressure.** An increase in blood pressure has been reported in women receiving oral contraceptives. The prevalence of hypertension in oral contraceptive users may be no higher than nonusers in the first year of oral contraceptive use but increases with longer exposure and in the fifth year of use is two and one-half to three times the reported prevalence in the first year. Women who previously had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given oral contraceptives. 9) **Headaches.** The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent or severe, requires discontinuation of oral contraceptives and investigation of the cause. 10) **Bleeding Irregularities.** Breakthrough bleeding, spotting and amenorrhea are frequent reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or change to another formulation may solve the problem. Changing to an oral contraceptive with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of oral contraceptives. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. 11) **Ectopic Pregnancy.** Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. However, in oral contraceptive failures, the ratio of ectopic to intrauterine pregnancies is higher than in women not using oral contraceptives since the drugs are more effective in preventing intrauterine rather than ectopic pregnancy. The higher ectopic-intrauterine ratio has been reported with both combination products and progestin-only oral contraceptives. 12) **Breast-Feeding.** A small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The long-range effect to the nursing infant cannot be determined at this time.

**Precautions:** 1) A complete medical and family history should be taken prior to the initiation of oral contraceptives. Examination should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, oral contraceptives should not be prescribed for longer than 1 year without another physical examination being performed. 2) Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3) Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. 4) Because oral contraceptives may cause some degree of fluid retention, conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, cardiac or renal insufficiency or asthma require careful observation. 5) Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving oral contraceptive therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6) Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7) Oral contraceptive users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8) Serum folate levels may be depressed by oral contraceptive therapy. This may complicate subsequent pregnancy with regard to folate deficiency. 9) The pathologist should be advised of oral contraceptive therapy when relevant specimens are submitted. 10) Certain endocrine and liver function tests and blood components may be affected by estrogen-containing oral contraceptives: a. Increased sulfobromophthalen retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin III; increased norepinephrine-induced platelet aggregability. c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T<sub>4</sub> by column, or T<sub>4</sub> by radioimmunoassay. Free T<sub>4</sub> resin uptake is decreased, reflecting the elevated TBG. Free T<sub>4</sub> concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test. 11) The active yellow tablets and the inert green tablets in Ovcon-50 (21 and 28 day regimens) and the inert green tablets in the 28 day regimen of Ovcon-35 contain FD&C Yellow No. 5 (tartrazine) which may cause allergic-type reactions (including bronchial asthma) in certain susceptible individuals. Although the overall incidence of FD&C Yellow No. 5 (tartrazine) sensitivity in the general population is low, it is frequently seen in patients who also have aspirin hypersensitivity.

**Information for the Patient:** Detailed Patient Labeling has been prepared for use by the patient and has been made available for distribution by the pharmacist.

**Drug Interactions:** Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

**Adverse Reactions:** An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see **Warnings**): Thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gall bladder disease, congenital anomalies. There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis; benign hepatomas; neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug related: Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10% or less of patients during the first cycle (or other reactions, as a general rule, are seen much less frequently or only occasionally); gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding; spotting; change in menstrual flow; dysmenorrhea; amenorrhea during and after treatment; temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes (tenderness, enlargement, and secretion); change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; changes in corneal curvature (steepening), intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives, and the association has been neither confirmed nor refuted: Premenstrual-like syndrome, catarracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria.

**Acute Overdosage:** Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

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# Amniostat-FLM: An initial clinical trial with both vaginal pool and amniocentesis samples

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Amniostat-FLM, a new rapid slide agglutination test, was compared with thin-layer chromatography as a method for detecting phosphatidylglycerol in amniotic fluid. This is the first reported use of Amniostat-FLM to evaluate vaginal pool and contaminated vaginal pool amniotic fluid. One hundred one of 161 amniotic fluid samples were collected from the vaginal pool. Thirty-nine of these were contaminated. Vaginal pool amniotic fluid, whether contaminated or not, did not adversely effect the ease of performance, reliability, or interpretation of Amniostat-FLM. This test seems ideally suited to institutions where 24-hour availability of thin-layer chromatography is not available. In institutions where it is available, Amniostat-FLM could be used as a screening test. Amniostat-FLM was found to be significantly less sensitive when compared with thin-layer chromatography in detecting the presence of phosphatidylglycerol. At our institution the screening of amniotic fluid samples with Amniostat-FLM before use of thin-layer chromatography was only cost effective at  $\geq 34$  weeks of gestation. (AM J OBSTET GYNECOL 1986;154:65-8.)

**Key words:** Amniostat-FLM, amniotic fluid, phosphatidylglycerol

Amniostat-FLM,\* a rapid, immunologic, semiquantitative slide test to assess the presence or absence of phosphatidylglycerol in amniotic fluid, was approved for clinical use in October, 1982. In November, 1983, Garite et al.<sup>1</sup> reported the use of this method with transabdominal amniocentesis specimens and compared it to a modified Gluck form of thin-layer chromatography and fluorescence polarization.

This kit method for assessing fetal pulmonary maturity seemed ideally suited to those institutions where high-risk obstetric care is given, but 24-hour availability of thin-layer chromatography for assessing pulmonary maturity is not possible. One objective of this research was to compare our laboratory's results with Amniostat-FLM to those reported by others.<sup>1,2</sup> Another objective was to apply the Amniostat-FLM kit to vaginal pool samples, including those contaminated with blood or meconium, since phosphatidylglycerol, unlike lecithin/sphingomyelin ratios, is not effected by vaginal contamination.<sup>3-5</sup>

## Material and methods

All samples of amniotic fluid obtained for this study were collected between March, 1983, and August, 1984,

and analyzed in the Clinical Chemistry Laboratory at the Maine Medical Center, Portland, Maine. Sixty fluids were collected by amniocentesis, and 101 were vaginal pool specimens. Each specimen was tested for the presence of phosphatidylglycerol with use of thin-layer chromatography and Amniostat-FLM. All specimens were centrifuged at 1000 rpm for 3 minutes and analyzed the same day or stored at  $-20^{\circ}\text{C}$  for future analysis.

The phosphatidylglycerol by thin-layer chromatography was determined by use of the commercially available kit, Helena Fetal-Tek 200.\* The manufacturer's procedure was followed exactly. In this method the phospholipids including lecithin, sphingomyelin, and phosphatidylglycerol were extracted from a sample with a chloroform-methanol mixture and streaked onto a thin-layer chromatography plate. They were separated by use of a solvent system containing chloroform, methanol, 2-propanol, triethylamine, and water. The phospholipids were made visible by use of a dilute cupric-acetate-phosphoric acid reagent and identified by comparison to a known marker. The phosphatidylglycerol was identified as present or absent.

The immunologic assay for phosphatidylglycerol was done by the commercially available kit Amniostat-FLM. Again the manufacturer's procedure was followed exactly. The procedure used was as follows: 25  $\mu\text{l}$  of sample and 25  $\mu\text{l}$  of an ethanol solution of lecithin and cholesterol were added to 250  $\mu\text{l}$  of a phosphate buffer solution. Then 25  $\mu\text{l}$  of an antibody solution to phos-

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*\*Amniostat-FLM is a product of Hana Biologics, Inc., Berkeley, California.*

*\*Helena Laboratories, Beaumont, Texas.*

**Table I.** Overall and individual method concordance and discordance rates for all gestational ages

	Amniocentesis		Vaginal pool		Overall	
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
Concordance	54/60	90	86/101	85.1	140/161	86.9
Discordance	6/60	10	15/101	14.9	21/161	13.1

**Table II.** Overall concordance and discordance rates for various gestational ages

	<34 wk		34 to 36 wk		>36 wk		Unknown gestational age	
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
Overall concordance	52/57	91.2	40/46	86.9	30/35	85.7	17/23	73.9
Overall discordance	5/57	8.8	6/46	13.1	5/35	14.3	6/23	26.1

**Table III.** Number of positive results for each method at various gestational ages

	<34 wk		34 to 36 wk		>36 wk	
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
Number-positive amniostat-FLM	6/57	10.5	15/46	32.6	24/35	68.6
Number-positive phosphatidylglycerol (thin-layer chromatography)	11/57	19.2	21/46	45.6	29/35	82.8

phatidylglycerol was placed into the well of a 7.5 × 3 cm glass slide provided with the kit. Ten microliters of thoroughly mixed sample suspension was added to the appropriate well on the slide. The slide was placed on a 60 rpm serologic rotator at room temperature for 9 minutes. All test samples were run with a positive and negative control. A positive result was indicated by the presence of large agglutinated particles with a clear background.

### Results

Amniostat-FLM and phosphatidylglycerol determination by thin-layer chromatography were completed on all 161 samples. Twenty-three samples were sent to our institution from outside centers, and 138 samples were obtained from our patients. Gestational ages ranged between 26 and 40 weeks, and the obstetric indications are as follows: premature rupture of membranes, preterm labor, pregnancy-induced hypertension, diabetes mellitus, intrauterine growth retardation, Rh isoimmunization, congenital anomaly, unknown gestational age, twins, and placenta previa. Amniostat-FLM and thin-layer chromatography were both negative in 85 samples and both positive in 55 samples. The 21 discordant results all were negative by Amniostat-FLM and positive by thin-layer chromatography.

The tests were considered concordant if Amniostat-FLM and thin-layer chromatography results were either both positive or both negative. Table I demonstrates that an overall concordance of 86.9% (140 of 161) was obtained if both amniocentesis and vaginal

pool samples were included. If just vaginal pool samples were studied, a concordance of 85.1% (86 of 101) was observed. The amniocentesis concordance of 90% (54 of 60) is very similar to that obtained by Garite et al.<sup>1</sup> and Lockitch et al.<sup>2</sup>

Table II shows concordance between Amniostat-FLM and the thin-layer chromatography plate for various gestational ages. At less than 34 weeks' gestation 57 samples were studied, with a concordance between both methods of 91.2% (52 of 57). Between 34 and 36 weeks the concordance was 86.9% (40 of 46). Beyond 36 weeks the observed concordance was 85.7% (30 of 35). Twenty-three samples of unknown gestational age had a concordance of 73.9% (17 of 23).

Positive tests by each method are compared at various gestational ages in Table III. Amniostat-FLM was positive 10.5% of the time (6 of 57) before 34 weeks' gestation, whereas the thin-layer chromatography method was positive 19.2% of the time (11 of 57). Between 34 and 36 weeks Amniostat-FLM was positive in 32.6% (15 of 46) of cases, while the plate was positive in 45.6% (21 of 46). For gestational ages of >36 weeks Amniostat-FLM and the plate had a percent positive rate of 68.6% (24/35) and 82.8% (29/35) respectively.

In addition to the 23 samples of amniotic fluid sent from outside institutions, we had seven patients who received antepartum care at our center but delivered elsewhere. This left us with 131 fluid-samples in which neonatal outcome data were available.

Twenty-seven cases of respiratory distress system occurred in the 131 neonates available for study. Am-



niostat-FLM was positive in 45 of these 131 samples. No cases of respiratory distress system occurred when Amniostat-FLM was positive. Phosphatidylglycerol by thin-layer chromatography was reported as present in 57 of the 131 samples. Of the 12 cases in which phosphatidylglycerol by thin-layer chromatography was present and Amniostat-FLM was negative, one case of respiratory distress system did occur. This false positive result was later explained when we modified the Helena system as described by Spillman et al.<sup>6</sup>

Our laboratory estimates that Amniostat-FLM costs \$35 and thin-layer chromatography \$100 per patient sample. Because no false positive results with use of Amniostat-FLM were observed, a protocol for handling each fluid sample was proposed whereby samples would be first screened with use of Amniostat-FLM and, if positive, would be reported as such without further thin-layer chromatography testing. Samples negative for Amniostat-FLM would be immediately followed with thin-layer chromatography. It was hoped that such a protocol would be acceptable, since laboratory technician time could be saved. Amniostat-FLM takes approximately 20 minutes to complete, whereas thin-layer chromatography takes 4 hours. The cost analysis in Table IV shows that  $\leq 33$  weeks' gestation it is not cost effective to use Amniostat-FLM as an initial test to screen amniotic fluid samples.

#### Comment

The purpose of this study was to compare our results with Amniostat-FLM to those already reported by Garite and Lockitch. We also wanted to evaluate this new semiquantitative kit on vaginal pool samples.

The 90% concordance rate with amniocentesis samples supports the 91% and 90% rates reported by Garite and Lockitch. It is interesting to note, however, that both of these authors found examples in which Amniostat-FLM was positive and phosphatidylglycerol by thin-layer chromatography was negative. The 21 discordant samples were all Amniostat-FLM negative and thin-layer chromatography positive. The particular thin-layer chromatography method used detects phosphatidylglycerol at low concentrations (positive at 0.5  $\mu\text{g/ml}$  of phosphatidylglycerol). It is therefore able to demonstrate more frequently the presence of phosphatidylglycerol at lower concentrations than is possible with the commercially available Amniostat-FLM kit (positive at 2  $\mu\text{g/ml}$  of phosphatidylglycerol).

The vaginal pool concordance rate (85.1%) is comparable with published amniocentesis data. Thirty-five of 101 vaginal pool samples were contaminated with blood. Stedman et al.<sup>4</sup> have reported the reliability of phosphatidylglycerol when detected in vaginal pool amniotic fluid, and Strassner et al.<sup>5</sup> have shown that blood contamination of amniotic fluid does not inter-

Table IV. Cost analysis

	$\leq 33$ wk	34-36 wk	$\geq 37$ wk
Positive Amniostat-FLM (\$35)	6/57	15/46	24/35
Positive thin-layer chromatography (\$100)	11/57	21/46	29/35
Proposed protocol*	\$1,995.00	\$1,610.00	\$1,225.00
	5,100.00	3,100.00	1,100.00
	\$7,095.00	\$4,710.00	\$2,325.00
Thin-layer chromatography alone	\$5,700.00	\$4,600.00	\$3,500.00

\*Proposed protocol: All samples tested first with Amniostat-FLM. Positive tests with use of Amniostat-FLM were not followed by thin-layer chromatography. Negative tests with use of Amniostat-FLM were followed by thin-layer chromatography.

fere with phosphatidylglycerol determination by thin-layer chromatography. Our study confirms this not only with use of thin-layer chromatography but also with Amniostat-FLM. Four vaginal pool samples were contaminated with meconium. There was 100% concordance between thin-layer chromatography and Amniostat-FLM for determination of phosphatidylglycerol in these meconium-stained samples; however, no conclusion can be drawn from this small number.

Amniostat-FLM has been shown to be predictive if positive for phosphatidylglycerol, rapid, easy to perform, easy to interpret, and inexpensive in studies with use of amniocentesis specimens. We feel that this statement can now be expanded to include vaginal pool samples of amniotic fluid, since the test does not appear to be adversely affected by sources of vaginal contamination.

All in all, Amniostat-FLM is a convenient kit method for semiquantitatively assessing the presence of phosphatidylglycerol in amniotic fluid. It is useful for both amniocentesis and vaginal pool samples and generally lives up to the claims made by its manufacturer. For these reasons it would seem that hospitals without 24-hour availability of thin-layer chromatography may benefit from use of such a test. Hospitals where 24-hour availability of thin-layer chromatography is available might benefit from use of Amniostat-FLM as a screening test for amniotic fluid samples of  $\geq 34$  weeks' gestation.

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## Use of the drop volume of amniotic fluid in estimating the risk for respiratory distress syndrome in the newborn infant

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The present study describes the testing and function of the drop-volume method in the analysis of fetal lung maturity with use of amniotic fluid. Elevated surface tension resulting from a lack of surface-active phospholipids (surfactant) is the primary etiologic defect in the development of respiratory distress syndrome. The drop-volume method quantifies the surface tension of amniotic fluid with use of the fact that the volume of a falling drop of liquid is proportional to the quantity of surfactant in the solution. The drop-volume method requires only 2 minutes and 2 ml of amniotic fluid and predicts fetal lung maturity with an accuracy equal to or greater than that of other tests currently in use. (AM J OBSTET GYNECOL 1986;154:68-74.)

**Key words:** Respiratory distress syndrome, fetal lung maturity, surface tension, amniotic fluid, surfactant

Deficiency of surface tension-lowering substances (surfactants) in the alveoli of the newborn baby is the primary etiologic factor in respiratory distress syndrome. The main source of surfactants in amniotic fluid is the fetal alveoli.

Respiratory distress syndrome, a severe disorder of the lungs of newborn infants, is responsible for more neonatal deaths than any other single disease.<sup>1</sup> Several investigators have found that a deficiency of surfactant in the lungs is the primary etiologic defect, causing elevated surface tension in the alveoli.<sup>1-3</sup> The physiologic significance of the surfactant for lung expansion has been shown in the rabbit with use of tracheal deposition of surfactant.<sup>4</sup>

A number of different methods for the prediction of respiratory distress syndrome have been described, most of them relying on direct measurement of the surfactant in the amniotic fluid by chemical assay. The lecithin/sphingomyelin ratio and the phosphatidylglycerol assay are the most widely used examples of this type of test. Determination of the lecithin/sphingo-

myelin ratio and phosphatidylglycerol values, however, is a relatively complicated test that requires a laboratory procedure of several hours.

The single rapid and inexpensive test currently available is the so-called shake test.<sup>5</sup> The shake test has several serious shortcomings; among them are the spurious results that may be caused by contamination of the specimen, widely variable standardization procedures, and a lack of precise quantification.

The development of a simple, reliable, and rapid test for fetal maturity would be an important addition to the clinical management of high-risk pregnancy. The present study is the description and application of a new device\* for measuring amniotic fluid surface tension derived from the drop-weight method of Harkins and Brown.<sup>6</sup>

### Material and methods

The Surfactometer drop-volume measurement evaluates the amount of surfactant present in the amniotic fluid with use of the fact that the volume of a falling drop of liquid is inversely proportional to the quantity of surface-active substances in the solution. Distilled water gives a value of 101.1 on the machine used in

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\*Amniotech RDS/100 Surfactometer, manufactured by Medical Equipment Designs, Inc., Laguna Hills, California.

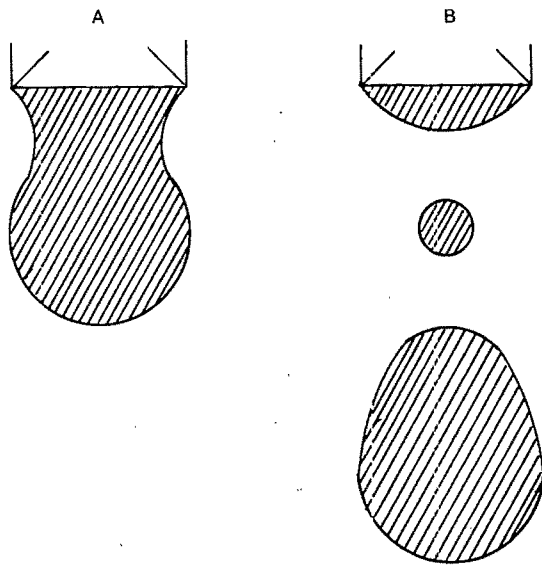


Fig. 1. Drop of liquid before (A) and after (B) it has left the drop-forming orifice.

the present study, whereas amniotic fluid from normal term pregnancies (>36 weeks of gestation) gives values of <85.0. The test requires 2 minutes of time and 2 ml of amniotic fluid.

**Theory.** In a simple model the drop volume is determined by the condition that the force of gravity on the drop equals the force of the surface tension around the circumference of the tip of the drop-forming orifice. This results in the following formula:

$$V \times \rho \times g = 2\pi r \times \gamma \quad (1)$$

where  $V$  = volume of drop,  $\rho$  = density,  $g$  = acceleration of gravity,  $r$  = radius of the tip, and  $\gamma$  = surface tension of liquid.

When strict accuracy is required, this formula is not sufficient because the surface of the drop at the edge of the tip is not vertical. Also the mass (or volume) of the falling drop is smaller than that of the hanging drop, since a fraction of the drop remains at the tip (Fig. 1, A and B). Therefore we cannot in theory compute the mass (volume) of the falling drop as a function of surface tension and tip radius. The following modification takes these factors into account:

$$V \times \rho \times g = 2\pi r \times \phi(r/L) \times \gamma \quad (2)$$

where  $\phi$  is an unknown function of the ratio  $r/L$ , and  $L$  is a linear measure of the size of the drop.

We take  $L$  to be equal to the cube root of  $V$  ( $L = V^{1/3}$ ), where  $V$  is the drop volume. Harkins and Brown<sup>6</sup> have, with great precision, experimentally determined this function. Thus a firm theoretical and experimental connection between drop volume and surface tension can be described by the following formula:

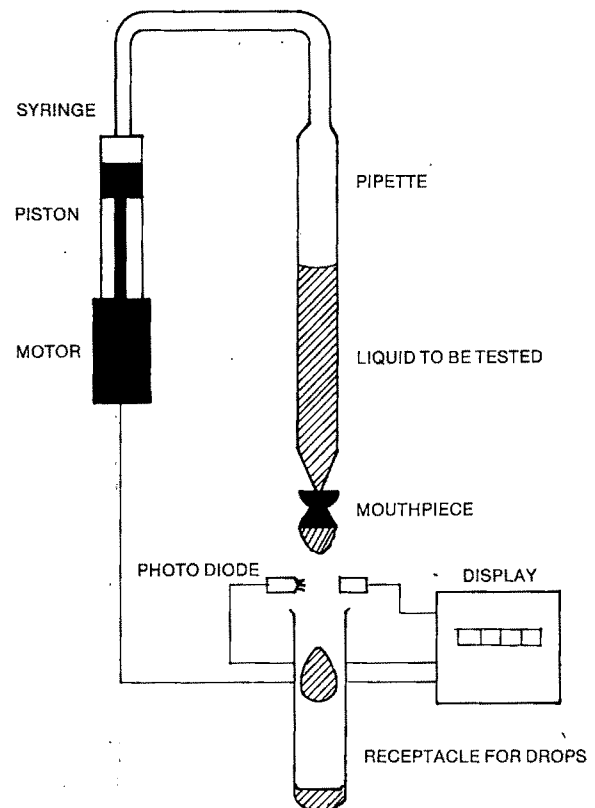


Fig. 2. An illustration of the design of the apparatus used for the measurement of amniotic fluid surface tension.

$$\gamma = V \times \rho \times g / 2\pi r \times \phi(r/v^{1/3}) \quad (3)$$

In practice,  $V$  is measured and used directly as an index of surface tension. This is possible because for formula 3, in the range of  $V$  for this test, there is an almost linear dependence between  $V$  and  $\gamma/\rho$  (surface tension/density). As density does not vary appreciably between different samples of amniotic fluid,  $V$  is a good measure of surface tension.

**Apparatus.** Test fluid is pushed through the tip of a vertically mounted narrow tube by a column of air (Fig. 2). The drops formed detach themselves from the tip and fall between a light-emitting diode and a photo-detector cell. The piston movement that controls the air column is controlled by a clock motor. The time between drops,  $S$ , is thus proportional to the drop volume.

**Patients.** One hundred seventy-three amniotic fluid specimens were obtained from patients enrolled in the prenatal clinic at University Hospital, Lund, Sweden, between January, 1984, and March, 1985. The specimens were collected by amniocentesis or by intrauterine catheter, and those specimens contaminated with meconium and/or blood were not used. The gestational age of the patients at the time of specimen collection varied between 26 and 43 weeks; 113 patients delivered shortly after their amniotic fluid was obtained (a period



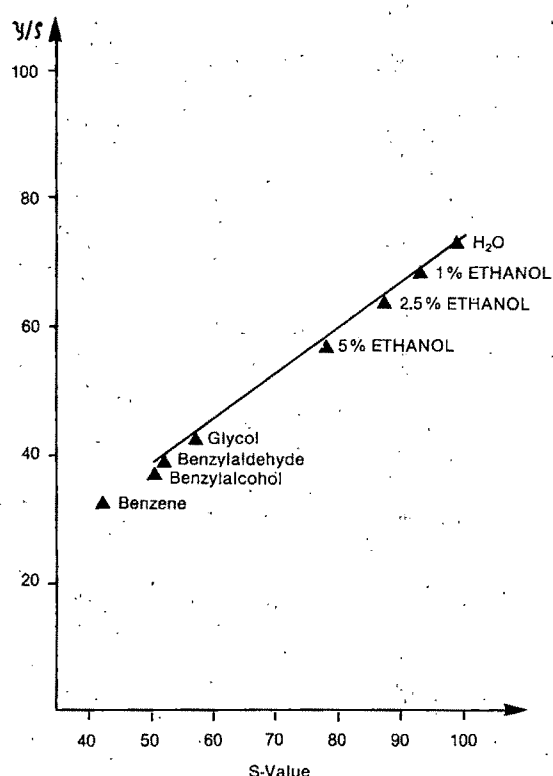


Fig. 3. The correlation between the surface tension/density ( $\gamma/\rho$ ) of eight test liquids with known values and their experimentally derived S values (▲).

of  $\leq 1$  week). The lecithin/sphingomyelin ratio results were obtained for five patients from this group. The S value of the 60 patients who did not deliver within 1 week was used only for comparison of their lecithin/sphingomyelin ratio test results.

The mean age of patients included in the study was 27 years. The mean parity was  $1.6 \pm 0.4$ , with 55% of the patients primiparous. There were no stillbirths and no multiple births in the study patient population.

## Results

**Validity of the method.** A series of experiments were performed to verify that the S value of the Surfactometer is a measure of the surface tension of amniotic fluid. S-value measurements were made for eight liquids with known values of  $\gamma$  and  $\rho$ , and the  $\gamma/\rho$  ratio was plotted against the S value for each test liquid (Fig. 3). The correlation curve was linear with very good agreement between the theoretical line and the experimentally derived values.

To determine whether variations in viscosity interfere with S-value measurements, the S value of water at different temperatures was tested. A climate chamber was used to maintain each of the six different temperatures that were evaluated. Viscosity is highly temperature dependent, and any disagreement between the theoretical and actual data points would become clear in such an experiment. The S values, however, lie

Table I. A comparison of S and T values for distilled water, 5% ethanol in water, and glycol

	S value	Tensiometer (T) (dyne/cm)	$S \times \rho/T$
Distilled water	$101.1 \pm 0.3$	$73.3 \pm 0.3$	$1.38 \pm 0.01$
5% Ethanol in water	$80.3 \pm 0.3$	$60.7 \pm 0.2$	$1.32 \pm 0.01$
Glycol	$58.8 \pm 0.4$	$50.4 \pm 12$	$1.30 \pm 0.29$

Values in column 3 are multiplied by  $\rho$  in order to compensate for density differences between the three fluid. The consistency of the corrected values in column 3 confirms that S value is a measure of  $\gamma/\rho$ .

on the curve predicted from the  $\gamma$  (surface tension) and  $\rho$  (density) temperature dependencies only (Fig. 4).

Comparison with a classical method for measuring surface tension—a du Nouy tensiometer—was made for distilled water, 5% ethyl alcohol, and glycol. The mean values and standard deviations are shown in Table I.

Seven different concentrations of ethyl alcohol in distilled water were tested empirically and found to be almost identical with the theoretically calculated S values (Fig. 5).

To test the effect of hemolyzed blood on surface tension, hemolyzed blood was added to distilled water in various concentrations. As expected, hemolyzed blood lowered the surface tension (Fig. 6). In contrast, fresh nonhemolyzed blood in amniotic fluid had no effect on the S value in concentrations up to 10% by volume (Fig. 7).

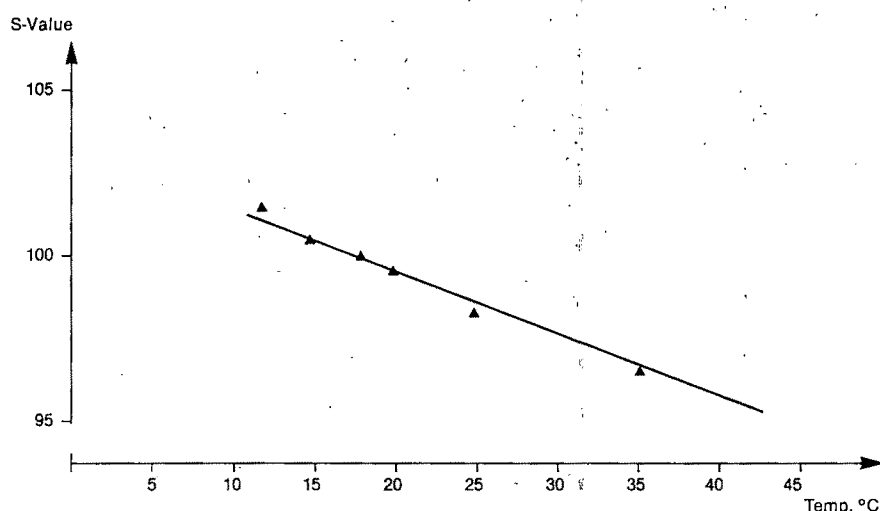
The S value of amniotic fluid contaminated with various concentrations of meconium was decreased (Fig. 8).

Several representative specimens of amniotic fluid were stored at 20°, 5°, and -10° C for a 2-week period with no effect on S values.

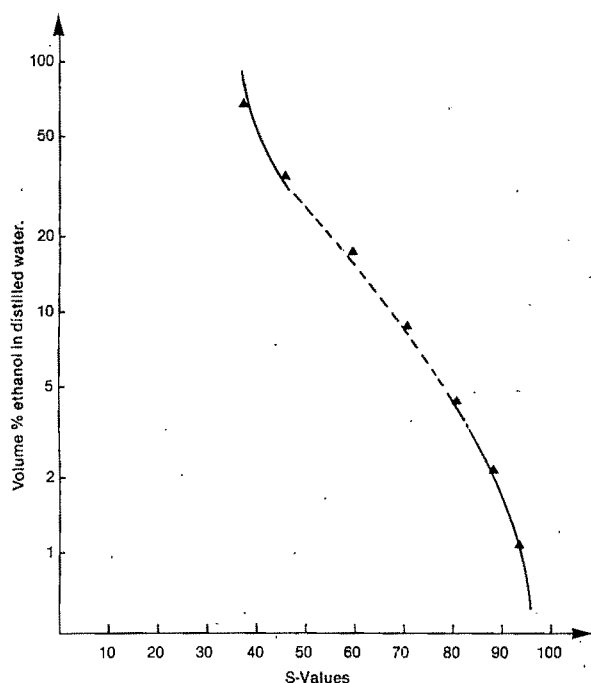
A dilution series of phosphatidylglycerol and phosphatidylglycerol plus dipalmitoyl phosphatidylcholine was prepared for the range of 12.5 to 0.02 mg/ml for phosphatidylglycerol and 30 to 0.06 mg/ml for dipalmitoyl phosphatidylcholine and tested for S values. The results are shown in Fig. 9. The steeper curve seen with phosphatidylglycerol plus dipalmitoyl phosphatidylcholine indicates that dipalmitoyl phosphatidylcholine has a surface tension-lowering effect above and beyond that of phosphatidylglycerol alone.

**Clinical testing.** For the 65 patients for whom a lecithin/sphingomyelin ratio was obtained, the comparison is shown in Table II. When the ratio was  $\geq 2.0$ , 46% of the S values were  $\leq 85.0$ . For cases with lecithin/sphingomyelin ratios of  $< 2.0$ , 76% of S values were  $> 85.0$ .

As shown in Table III, of the 113 infants who were delivered within 1 week of specimen collection, 11 developed respiratory distress syndrome (9.7%). Of these

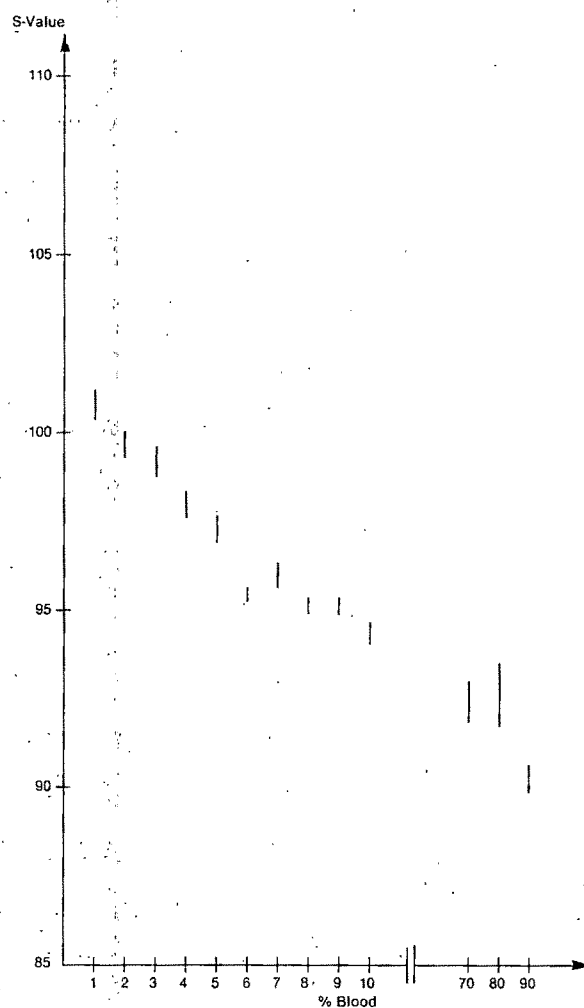


**Fig. 4.** The theoretical curve and empirically observed ( $\blacktriangle$ ) S values of distilled water at different temperatures. The curve is based upon the theoretical surface tension/density ( $\gamma/\rho$ ). If viscosity influenced or interfered with the S value, the data points would deviate from the line predicted with use of  $\gamma/\rho$  alone.



**Fig. 5.** Comparison between the expected curve and experimentally derived ( $\blacktriangle$ ) S values of different concentration of ethanol in distilled water.

11 cases, nine were predicted on the basis of S value. Of the two cases of respiratory distress syndrome that were not predicted by the Surfactometer, one infant suffered from hemolytic disease of the newborn and required exchange blood transfusion, and the other developed sepsis. Both of these factors are known to increase the risk of respiratory distress syndrome despite the presence of adequate surfactant. For the 11 infants who developed respiratory distress syndrome,



**Fig. 6.** A graphic plot of the S values of distilled water with various percentages of added blood (hemolyzed blood). Values shown represent the mean  $\pm$  SD for 10 readings of each concentration.

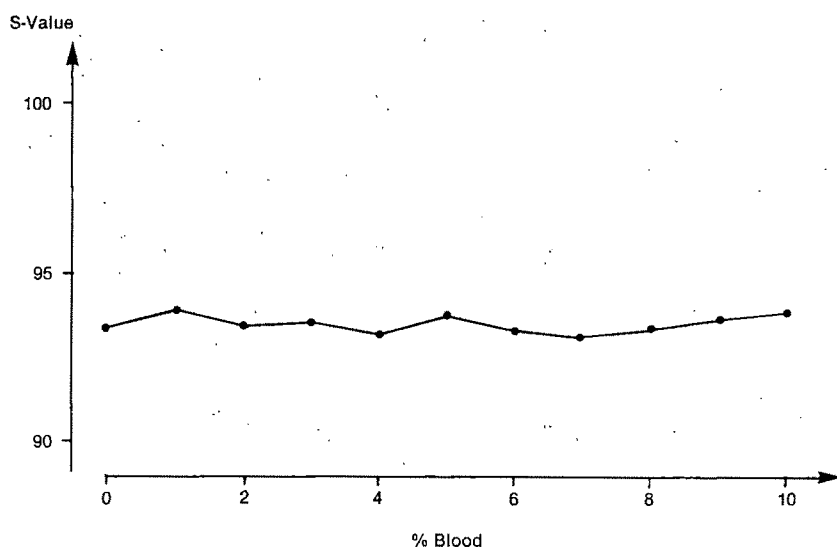


Fig. 7. A graphic plot of the S values of amniotic fluid with various percentages of added blood (nonhemolyzed). Values shown represent the mean of 10 readings.

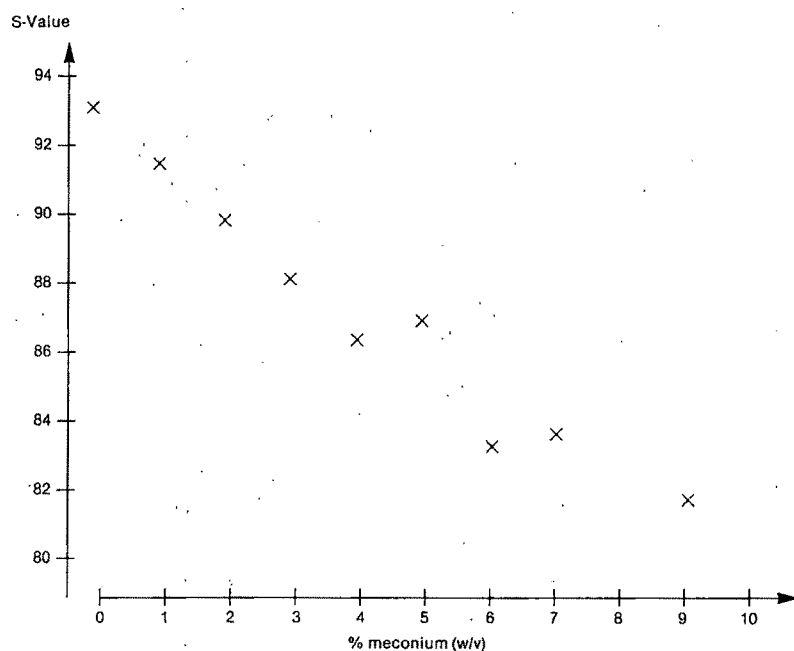


Fig. 8. A graphic plot of the S values of amniotic fluid with various concentrations of added meconium.

the mean S value was  $88.2 \pm 2.4$ . The mean S value for those infants who did not develop respiratory distress syndrome was  $81.0 \pm 3.6$ .

The results shown in Table III indicate that an S value of  $\leq 85.0$  is associated with a healthy infant 98% of the time (negative predictive value). Furthermore, 92% of the healthy infants in the study series were predicted by the S value (specificity); 82% of the 11 cases in which respiratory distress syndrome developed were predicted by an S value of  $\geq 85.1$ . Of those infants with an S value of  $\geq 85.1$ , 53% developed respiratory

distress syndrome (positive predictive value). The results shown in Table III compare very favorably with similar results computed for the lecithin/sphingomyelin ratio. For the two most important components, positive and negative predictive value, the S value yields nearly identical results (53% versus 54% and 98% versus 98%, respectively).<sup>7</sup>

#### Comment

From the results illustrated in Fig. 3, we have established the clear linear relationship of S value to surface



**Table II.** A comparison between the lecithin/sphingomyelin (L/S) ratio and S values of the amniotic fluid of 65 patients

	$S \leq 85.0$	$S \geq 85.1$	Total
L/S ratio $\geq 2.0$	22	26	48
L/S ratio $< 2.0$	4	13	17
Total	26	39	65

**Table III.** A comparison between the S values and neonatal outcome for 113 cases with deliveries within 1 week after amniotic fluid specimen collection

	Respiratory distress syndrome (+)	Respiratory distress syndrome (-)	Total
$S \geq 85.1$	9	8	17
$S \leq 85.0$	2	94	96
Total	11	102	113

Sensitivity for respiratory distress syndrome (+):  $9/11 = 82\%$ .

Specificity for respiratory distress syndrome (+):  $94/102 = 92\%$ .

Positive predictive value:  $9/17 = 53\%$ .

Negative predictive value:  $94/96 = 98\%$ .

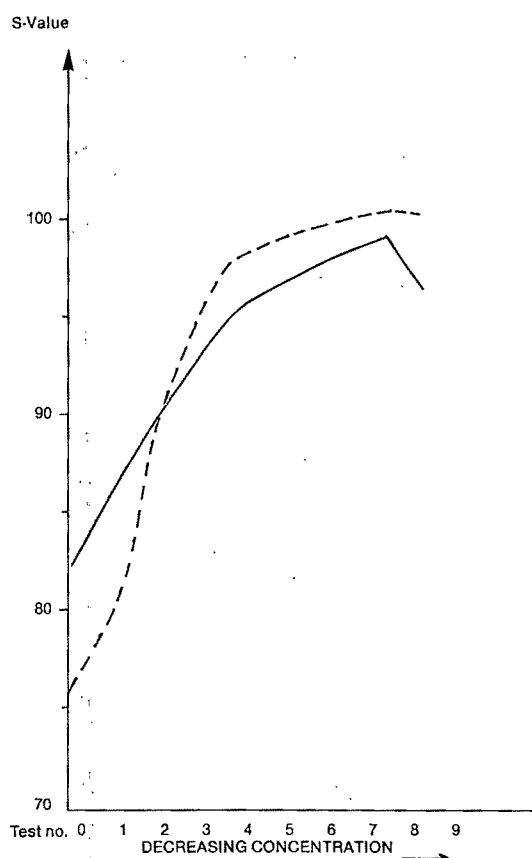
tension, thus showing the drop-volume method to be a reliable index of surface tension.

The results of experiments on liquids of known surface tension confirm that the drop-volume method is a reliable index for measuring surface tension. The correspondence of S value to tensiometer results is nearly exact. Furthermore, experiments with known concentrations of phosphatidylglycerol and phosphatidylglycerol plus dipalmitoyl phosphatidylcholine (Fig. 9) demonstrate the sensitivity of the drop-volume method to the presence of surface-active phospholipids.

The drop-volume method directly measures the total surfactant activity of the amniotic fluid, thus providing a theoretical advantage over other methods that evaluate only one of the surface-active substances in amniotic fluid. In fact, as the results in Table III indicate, the S value compares very well with the lecithin/sphingomyelin ratio with respect to positive and negative predictive value.

The presence of hemolyzed blood or of meconium interferes with the results of the drop-volume test by lowering the surface tension and thus decreasing the S value. When one or both of these substances is present in the amniotic fluid, the results are not as reliable and must be interpreted with caution. Fresh blood, however, up to a concentration of 10% does not significantly affect S value.

The tests of fetal lung maturity which are in widespread use—the lecithin/sphingomyelin ratio and the



**Fig. 9.** A graphic plot of the dilution curves of distilled water with decreasing concentrations of phosphatidylglycerol (solid line) and phosphatidylglycerol plus dipalmitoyl phosphatidylcholine (dotted line).

assay for phosphatidylglycerol—are time consuming and expensive and require specialized laboratory techniques. In addition, each of these tests measures only one of the surface-active phospholipids present in amniotic fluid. The drop-volume method represents a simple, rapid, and inexpensive test that reliably detects fetal lung maturity with 98% accuracy. The test is very rapid and requires only 2 ml of amniotic fluid, and can be performed at the bedside without the need for specialized laboratory procedures or personnel.

In conclusion, the drop-volume method offers several clear advantages when compared to the spectrum of tests for fetal lung maturity which are currently in use.

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## Fetoplacental steroid metabolism in prolonged pregnancies

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The response to an intravenous load of 50 mg of dehydroepiandrosterone sulfate given to women with a pregnancy prolonged to more than 42 weeks was compared to the response in control pregnant women at 40 weeks. The half-life of dehydroepiandrosterone sulfate was longer in the prolonged pregnancy group than in the control group (mean  $\pm$  SEM,  $3.64 \pm 0.24$  hour versus  $2.78 \pm 1.08$  hour,  $p < 0.05$ ), and the rises of serum free estrone and free estradiol 4 hours after infusion were less in the prolonged pregnancy group than in the control group. Maternal venous and umbilical venous estrone, estradiol, free estriol, and dehydroepiandrosterone sulfate levels were compared in samples from control, postmature, and postterm groups. Umbilical estriol concentrations were significantly less in the postmature group ( $67.8 \pm 9.5$  ng/ml, mean  $\pm$  SEM) than in the control group ( $136 \pm 22.8$  ng/ml, mean  $\pm$  SEM,  $p < 0.01$ ), but there were no significant differences between dehydroepiandrosterone sulfate, estrone, and estradiol levels. Maternal venous estriol levels were lower in the postmature group ( $13.3 \pm 2.1$ ,  $p < 0.05$ ) than in the control group ( $25.0 \pm 4.9$ ). A reduction in overall placental estrogen production was indicated by the results of the dehydroepiandrosterone sulfate loads in the patients with prolonged pregnancy, yet the normal umbilical venous estrone and estradiol levels do not fit this conclusion. There is no explanation for the discrepancy at this time. (*AM J OBSTET GYNECOL* 1986;154:74-9.)

**Key words:** Dehydroepiandrosterone sulfate loads, postmature, postterm, placental steroids, fetal steroids

In a previous study from this laboratory,<sup>1</sup> differences were found in the fetoplacental steroid metabolism of fetuses of more than 42 weeks' gestation with the postmaturity syndrome (postmature fetuses) as compared with fetuses of greater than 42 weeks who showed no signs of dysmaturity (postterm fetuses). Levels of unconjugated estriol in umbilical venous blood were significantly less in the postmature fetuses than in the postterm fetuses, but levels of dehydroepiandrosterone sulfate were similar in these same umbilical samples. Evaluation of adrenocortical function in the first days of life in these two groups of infants showed similar responses of serum dehydroepiandrosterone sulfate and cortisol to adrenocorticotrophic hormone stimulation. Thus the low umbilical venous estriol levels in

postmature fetuses were not obviously secondary to reduced fetal adrenal secretion of the major estrogen precursor dehydroepiandrosterone sulfate. Rather there was a possibility that underactivity of the placental enzymes involved in the conversion of neutral steroid sulfates to estrogens could be responsible for the low estriol concentrations found.

This study focused on several approaches to the evaluation of placental conversion of neutral steroid sulfates to estrogens in prolonged pregnancies of more than 42 weeks' duration. Free estrone, free estradiol, free estriol, and dehydroepiandrosterone sulfate levels were assayed in a series of matched maternal venous and umbilical venous blood samples from control pregnancies and prolonged pregnancies. Intravenous loads of dehydroepiandrosterone sulfate (50 mg) were administered to a group of control women at term and to a group of women with prolonged pregnancies before onset of labor. Peripheral venous levels of estrone, estradiol, and dehydroepiandrosterone sulfate were measured after the dehydroepiandrosterone sulfate loads in order to estimate the pattern of conversion of

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dehydroepiandrosterone sulfate to the estrogens and to estimate the half-life of dehydroepiandrosterone sulfate. The results are compatible with the conclusion that placental conversion of neutral steroids to estrogens is lessened in the prolonged pregnancies.

### Material and methods

**Patients.** All pregnant women and newborn infants studied in this investigation were hospitalized in the Oregon Health Sciences University Hospital. A postmature infant was defined as an infant born at greater than 42 completed weeks' gestation with evidence of dysmaturity, and a postterm infant as an infant born at more than 42 weeks' gestation but with no physical signs of dysmaturity. Clifford<sup>2</sup> classified the dysmature signs of postmaturity into three stages, with the Stage 1 infant showing loss of vernix, skin maceration, malnutrition with fat loss, and an open-eyed and alert appearance. An infant with Stage 2 signs had, in addition to Stage 1 signs, a meconium-filled amniotic sac, meconium-covered skin and placental membranes, and evidence of fetal distress or anoxia. Stage 3 infants showed all of the above signs and, in addition, a dirty yellow cord and evidence of chronic asphyxia. The infants classified as postmature in this study were mainly Stage 1, and none were Stage 3. Infants classified as postterm had, at the most, mild peeling of the skin and terminal meconium at delivery, with no evidence for fat loss. No infants were included who had multiple congenital malformations or congenital viral illnesses.

**Dehydroepiandrosterone sulfate loads.** A group of women who were attending the Obstetrics Clinic at The Oregon Health Sciences University and who were identified as having a fetus of more than 42 weeks' gestation on the basis of a good history and early physical findings and/or two ultrasound examinations between 20 and 34 weeks were recruited into a study of serum steroid responses to a dehydroepiandrosterone sulfate load. The women were not in labor. They were studied on an outpatient basis, before performance of a contraction stress test, or as inpatients prior to the induction of labor or performance of a cesarean section for indications of abnormal fetal status. The study was approved by The Oregon Health Sciences University Committee on Human Research, and informed consent was obtained in each case. The dehydroepiandrosterone sulfate load was carried out in the following manner: Crystalline dehydroepiandrosterone sulfate (Schwartz-Mann Laboratories) was recrystallized twice from ethanol and methanol. A 50 mg aliquot of the dried dehydroepiandrosterone sulfate crystals, just prior to use, was dissolved in 10 ml of sterile 0.9% saline solution United States Pharmacopeia containing 5% ethanol. The solution was administered intravenously through a 0.22  $\mu$ m Millipore filter during 5 minutes. A short catheter was placed in the opposite arm, prior

to the dehydroepiandrosterone sulfate injection, for use in drawing the blood samples. Blood samples of 5 ml were drawn before and 30, 60, 120, 180, and 240 minutes after completion of the injection. The estrone, estradiol, estriol, and dehydroepiandrosterone sulfate concentrations were assayed in each blood sample. The dehydroepiandrosterone sulfate loads were carried out in 21 women with gestations of more than 42 completed weeks. Four of the infants born of these pregnancies were diagnosed by one of the authors (J. W. R.) as postmature and 17 were diagnosed as postterm. An umbilical venous blood sample was obtained from each infant and estrone, estradiol, estriol, and dehydroepiandrosterone sulfate levels were assayed.

A control group of 11 women was recruited from women admitted at term to the Oregon Health Sciences University Hospital for elective delivery by cesarean section before the onset of labor. The operative deliveries were done because of a previous cesarean section with the women not electing vaginal delivery. The women had weight gains of 20 to 35 pounds, did not smoke, did not drink more than 1 ounce of ethanol per week, had normal blood pressure, and were otherwise well with no chronic medication intake. A 50 mg load of dehydroepiandrosterone sulfate, as described above, was administered to each woman late on the day before the cesarean section. Blood samples were drawn as outlined in the paragraph above, and an umbilical venous blood sample was obtained from each of the infants. All infants were normally grown and had no anomalies, infections, or signs of dysmaturity. The study of the control women was approved by The Oregon Health Sciences University Committee on Human Research and informed consent was obtained in each case.

**Maternal venous-umbilical venous paired specimens.** In addition to the 21 preload maternal serum and matching umbilical venous serum samples obtained from the women with prolonged gestations and receiving dehydroepiandrosterone sulfate loads, 54 maternal venous samples were obtained from other women at more than 42 weeks' gestation who refused a dehydroepiandrosterone sulfate load, who could not be suitably scheduled for a dehydroepiandrosterone sulfate load, or who entered the hospital in labor. All maternal samples were obtained within 12 hours of delivery. Umbilical venous samples were obtained from the infant of each woman sampled. Of the 54 pregnancies, 18 newborn infants were diagnosed by one of the authors (J. W. R.) to be postmature and 36 were considered to be postterm. The estrone, estradiol, estriol, and dehydroepiandrosterone sulfate levels were measured in each maternal and umbilical venous serum sample.

### Steroid assay procedures

**Dehydroepiandrosterone sulfate.** The procedure for the radioimmunoassay of dehydroepiandrosterone sulfate



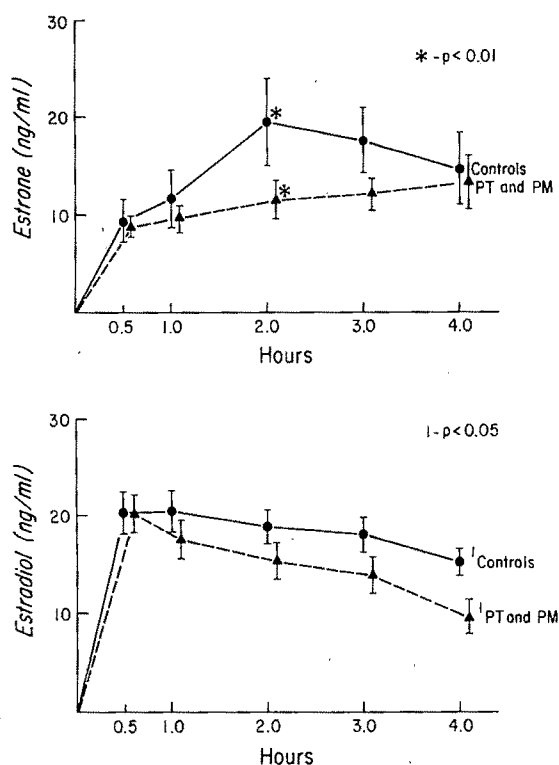


Fig. 1. The increases in maternal serum estrone and estradiol after 50 mg dehydroepiandrosterone sulfate loads to control women and a combined group of women carrying postterm (PT) and postmature (PM) fetuses.

was that described by Turnipseed et al.,<sup>3</sup> except that dextran-coated charcoal was used to separate bound from free steroid.

**Unconjugated estriol.** The procedure was that described by Barnhart et al.<sup>1</sup>

**Unconjugated estrone.** Serum samples were extracted with benzene. An anti-estrone-6-(CMO) bovine serum albumin antibody E001 from Steranti Research Ltd. was used in a 1:1000 dilution, and dextran-coated charcoal was used for bound and free steroid separation. The cross-reactivities of the antibody (estrone, 100%) were: 17 $\beta$ -estradiol, 0.1%; estriol, 0.01%; dehydroepiandrosterone, 0.01%; progesterone, <0.01%. Intra-assay variability was 2.1%, and interassay variability was 13.2%.

**Unconjugated estradiol.** Serum samples were extracted with benzene. A rabbit antiestradiol antibody (R-14), provided by Dr. L. Don Keith, Veteran's Administration Hospital, Portland, Oregon, was used in the radioimmunoassay procedure. The antibody was used in a 1:400,000 dilution and dextran-coated charcoal was used for bound and free steroid separation. The cross-reactivities of the antibody (17 $\beta$ -estradiol, 100%) were: estrone, 2.0%; estriol, 0.2%; dehydroepiandrosterone, 0.01%; testosterone, 0.06%; cortisol, 0.03%. Intra-assay variability was 7.0%, and interassay variability was 13.0%.

## Results

**Dehydroepiandrosterone sulfate loads.** The results in the four women who were delivered of postmature infants were not significantly different from the results in women who were delivered of postterm infants. Thus the data from the 21 women with gestations greater than 42 weeks were combined.

**Dehydroepiandrosterone sulfate falloff.** The data from 10 of the 11 control patients were complete enough to allow calculation of the dehydroepiandrosterone sulfate half-life. The half-life in the control patients was  $2.78 \pm 1.08$  hour (mean  $\pm$  SEM). The half-life in the 21 patients with prolonged pregnancy was  $3.64 \pm 0.24$  hour (mean  $\pm$  SEM), significantly longer than that of the control group ( $p < 0.05$ ). The half-life values were calculated by the method of least squares.

**Rise in serum estrone and estradiol levels.** The values of the absolute increases in estrone over pre-dehydroepiandrosterone sulfate load values are listed in Table I and shown in Fig. 1. The peak mean estrone value in the control patients was at 2 hours, while the patients with prolonged pregnancy showed a steady rise in mean estrone levels up to 4 hours. The mean estrone levels in the two patient groups were significantly different at 2 hours ( $p < 0.01$ ). A 2 by 5 analysis of variance was carried out and significant F values were obtained for time ( $F = 6.79$ ;  $df = 4, 110$ ;  $p < 0.01$ ) and the group by time interaction ( $F = 3.36$ ;  $df = 4, 110$ ;  $p < 0.05$ ).

The values of the absolute increases in estradiol over pre-dehydroepiandrosterone sulfate load values are listed in Table II and shown in Fig. 1. The mean peak estradiol values in both groups were at 0.5 hour. The mean values in each declined steadily thereafter, becoming significantly different at 4.0 hours ( $p < 0.05$ ). A 2 by 5 analysis of variance was performed on these data and significant F values for time ( $F = 37.10$ ;  $df = 4, 104$ ;  $p < 0.01$ ) and group by time interaction ( $F = 2.92$ ;  $df = 4, 104$ ;  $p < 0.05$ ) were obtained.

**Maternal venous and umbilical venous paired specimens.** In Table III are listed the serum levels of estrone, estradiol, estriol, and dehydroepiandrosterone sulfate in the maternal venous serum samples. The preload values of the control women are listed, and the preload values of the women delivered of postterm and postmature infants are combined with the samples from women with prolonged gestations not receiving dehydroepiandrosterone sulfate loads. The estriol level of  $13.3 \pm 2.1$  ng/ml (mean  $\pm$  SEM) in the postmature group is significantly less than the estriol level of  $25.0 \pm 4.9$  ng/ml in the control group ( $p < 0.05$ ).

The serum levels of estrone, estradiol, estriol, and dehydroepiandrosterone sulfate in the umbilical venous samples are listed in Table IV. The values from infants born to women with prolonged pregnancies receiving dehydroepiandrosterone sulfate loads are com-

**Table I.** Dehydroepiandrosterone sulfate loads: Absolute increase in estrone

Group	Increase in estrone (ng/ml)				
	0.5 hr	1.0 hr	2.0 hr	3.0 hr	4.0 hr
Control (n = 11)	9.4 ± 1.5	14.4 ± 3.8	21.1 ± 4.9*	16.9 ± 2.9	15.3 ± 3.2
Postterm and postmature	8.3 ± 0.9 n = 18	9.1 ± 1.2 n = 20	10.9 ± 1.7* n = 20	12.1 ± 1.6 n = 19	13.3 ± 2.5 n = 17

Values are mean ± SEM.

\*p &lt; 0.01.

**Table II.** Dehydroepiandrosterone sulfate loads: Absolute increase in estradiol

Group	Increase in estradiol (ng/ml)				
	0.5 hr	1.0 hr	2.0 hr	3.0 hr	4.0 hr
Control (n = 10)	21.1 ± 1.7	19.9 ± 1.8	18.6 ± 1.4	18.5 ± 1.4	15.0 ± 1.4*
Postterm and postmature	20.4 ± 1.8 n = 18	17.2 ± 1.7 n = 19	15.3 ± 1.6 n = 19	13.9 ± 1.7 n = 18	9.6 ± 1.5* n = 17

Values are mean ± SEM.

\*p &lt; 0.05.

**Table III.** Maternal venous samples

Group	n	Estrone (ng/ml)	Estradiol (ng/ml)	Estriol (ng/ml)	Dehydroepiandrosterone sulfate
Control	11	15.2 ± 2.6	26.5 ± 2.9	25.0 ± 4.9*	595 ± 119
Postterm	53	14.3 ± 0.9	27.9 ± 1.2	16.7 ± 1.4	880 ± 71.2
Postmature	22	14.1 ± 1.5	26.7 ± 2.3	13.3 ± 2.1*	1081 ± 193

Values are mean ± SEM.

\*p &lt; 0.05.

**Table IV.** Umbilical venous samples

Group	n	Estrone (ng/ml)	Estradiol (ng/ml)	Estriol (ng/ml)	Dehydroepiandrosterone sulfate
Control	11	32.1 ± 6.4	13.7 ± 1.7	136.5 ± 22.8*	2034 ± 220
Postterm	53	48.1 ± 3.8	16.7 ± 1.2	105.3 ± 12.3	2232 ± 157
Postmature	22	40.0 ± 4.9	17.1 ± 1.6	67.8 ± 9.5*	2063 ± 167

Values are mean ± SEM.

\*p &lt; 0.01.

bined with umbilical samples from the women with prolonged pregnancies and no dehydroepiandrosterone sulfate and are divided into postterm and postmature groups. The combining of values from infants of dehydroepiandrosterone sulfate-loaded and nonloaded mothers was justified by a comparison of the umbilical steroid values, which revealed no consistent differences between infants in the two groups, and by labeled tracer studies of dehydroepiandrosterone sulfate disposition in human pregnancy, which have shown that <1% of administered dehydroepiandrosterone sulfate reaches the fetus as either dehydroepiandrosterone sulfate or one of its metabolites.<sup>4,5</sup> The estriol value of 67.8 ± 9.5 ng/ml (mean ± SEM) in the postmature group is significantly less (p < 0.01) than the umbilical venous estriol level of 136 ± 22.8 ng/ml in the control group.

Because of unequal variances, the Kruskal-Wallis test was used to test for the significance of the differences.

### Comment

An interest in steroid metabolism of the fetoplacental unit of women with prolonged pregnancies has come from attempts to find a way to make an early diagnosis of fetal jeopardy, in time to allow the safe delivery of the infant. The 24-hour urinary estriol excretions were used in the past,<sup>6</sup> and more recently estrogen:creatinine ratios in single voidings<sup>7</sup> and serum free estriol levels<sup>8,9</sup> have been used to judge fetal health. Although biophysical means of judging fetal health in prolonged pregnancies are heavily relied on,<sup>10,11</sup> 37.5% of academic institutions continue to carry out estriol deter-

minations as part of their management of postterm pregnancies.<sup>12</sup>

Low estriol production by the fetoplacental unit could be due to fetal adrenocortical hypofunction with a decrease in production of neutral steroid estriol precursors, an underactivity of placental enzymatic conversion of neutral steroid precursors to estriol, or a combination of these two possibilities. A previous investigation from this laboratory indicated that adrenocortical function in postmature newborn infants was not different from that in postterm infants of the same gestational age and suggested that placental conversion of neutral steroid precursors to estriol might be abnormally decreased.<sup>1</sup> Thus one might expect to find a reduction in the other estrogens, estrone and estradiol, in situations where estriol levels are reduced.

The umbilical venous estriol levels shown in Table IV are significantly lower in the postmature fetuses than in the control fetuses, while dehydroepiandrosterone sulfate levels in the two groups are similar. This finding confirms that reported previously<sup>1</sup> and again points to a placental rather than a fetal cause of low estriol production in postmature infants. That the estriol production in the postmature pregnancies studied was indeed low by standard criteria is shown in Table III by the significantly lower estriol levels in maternal venous samples from the women carrying the postmature fetuses than in the venous samples from the control women. The mean maternal venous free estriol concentration of 13.3 ng/ml in the postmature group is similar to the mean level of 13.7 ng/ml reported by Gauthier et al.<sup>8</sup> in the forty-third week of gestation and is in the abnormal range of Yeh and Read.<sup>9</sup>

In contrast to what was anticipated, the estrone and estradiol concentrations in the umbilical venous samples from the postmature fetuses were not different from concentrations in the control infants (Table IV). Thus, on the basis of the fetal samples, there was no clear evidence for a general reduction in activity of the placental enzymes in the conversion of neutral steroids to estrogens.

However, the results of the dehydroepiandrosterone sulfate loads administered to the women in the control and prolonged pregnancy groups showed that there were significant differences between the groups in the absolute increases in estrone and estradiol in response to the dehydroepiandrosterone sulfate loads, as measured by analysis of variance (Tables I and II and Fig. 1). The pattern of rise of estrone and estradiol in the control women, the estradiol peak at 30 minutes, and the estrone peak at 120 minutes or later are consistent with the findings of other investigators who also have used the dehydroepiandrosterone sulfate load as a dynamic test of placental function.<sup>13-16</sup> The findings in this study, of lower absolute increases of estrone and estra-

diol and a late appearance of the estrone peak in the prolonged gestation group, are similar to the observations of others in women with preeclampsia and with growth-retarded fetuses.<sup>14-16</sup> The falloff rate of dehydroepiandrosterone sulfate after an intravenous load also has been a measure of placental dehydroepiandrosterone sulfate clearance and thus placental function. It has been used principally as a means of evaluating women with growth-retarded fetuses,<sup>17, 18</sup> and significantly prolonged dehydroepiandrosterone sulfate half-lives have been found in such cases. The finding in this study of a prolonged mean half-life of dehydroepiandrosterone sulfate as well as a reduced conversion of dehydroepiandrosterone sulfate to estrone and estradiol in the prolonged gestation group, as compared with the control group, indicates that placental metabolism of dehydroepiandrosterone sulfate also is limited in prolonged pregnancies.

Reduced placental clearance of dehydroepiandrosterone sulfate has been attributed to disturbances of uteroplacental blood flow,<sup>19</sup> and such abnormalities are features of preeclampsia and fetal growth retardation, the clinical abnormalities that have been most frequently described to be associated with prolonged dehydroepiandrosterone sulfate falloff rates and decreased conversions of dehydroepiandrosterone sulfate loads to estrone and estradiol. In addition, changes primarily in the vascular system, indicating abnormalities in uteroplacental blood flow, are considered to be the defining features of the aging process in placentas of prolonged pregnancies.<sup>20</sup> Thus in prolonged pregnancies, where uteroplacental blood flow is reduced as a result of the pathologic process, the placental role in dehydroepiandrosterone sulfate clearance and estrogen biosynthesis from dehydroepiandrosterone sulfate may be limiting in proportion to the decrease in placental perfusion. The extent of a decrease in steroid metabolizing enzymes in placentas from prolonged pregnancies as a result of the vascular disease is not known, as *in vitro* studies of steroid enzyme activities in these placentas have not been reported.

The discrepancy between the decreased aromatizing function of the placentas in the prolonged gestation group, indicated by the results of the maternal dehydroepiandrosterone sulfate loads, and the normal umbilical venous estrone and estradiol levels is not explained. It is possible that the loading of the aromatizing enzyme system by the dehydroepiandrosterone sulfate infusions in the pregnant women brought out a diminished capacity that was not evident in the resting fetal estrone and estradiol concentrations. The significantly low fetal serum estriol levels in association with the normal estrone and estradiol levels may also be explained by studies that suggest that 16 $\alpha$ -hydroxydehydroepiandrosterone sulfate, the neutral steroid



precursor of estriol, is a less efficient substrate for placental aromatizing enzymes than is dehydroepiandrosterone sulfate, the precursor of estrone and estradiol.<sup>21</sup> Another possible explanation for the low fetal estriol levels, in the face of normal dehydroepiandrosterone sulfate, estrone, and estradiol levels in the postmature group, is a reduced fetal hepatic 16 $\alpha$ -hydroxylase activity for dehydroepiandrosterone sulfate substrate, leading to a reduced fetal circulating 16 $\alpha$ -hydroxydehydroepiandrosterone sulfate concentration. However, these latter possibilities have not been examined in cases of prolonged gestation.

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# Plasma catecholamine responses to physiologic stimuli in normal human pregnancy

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The dynamic response of the sympathoadrenal system was evaluated during and after pregnancy in 13 healthy women with a protocol that compared cardiovascular parameters and plasma catecholamine levels during the basal state, after postural maneuvers, and following isometric exercise. Plasma epinephrine and norepinephrine levels were similar during and after gestation when the women rested on their sides, but heart rate was greater in pregnancy. Ten minutes of supine recumbency produced minimal changes, but attenuation of the anticipated increases in heart rate and plasma norepinephrine levels during standing and isometric exercise were observed during pregnancy. In contrast, alterations in plasma epinephrine appeared unaffected by gestation. Plasma renin activity and aldosterone levels were, as expected, greater during pregnancy; however, increments in response to upright posture were similar in pregnant and postpartum women. To the extent that circulating catecholamines may be considered indices of sympathoadrenal function, these data suggest that normal pregnancy alters cardiovascular and sympathetic nervous system responses to physiologic stimuli. (AM J OBSTET GYNECOL 1986;154:80-4.)

**Key words:** Norepinephrine, epinephrine, posture, isometric exercise, cardiovascular responses

The sympathetic nervous system plays a role in the control of blood pressure in normotensive nonpregnant subjects and may contribute significantly to elevated blood pressure in certain forms of human and experimental hypertension.<sup>1</sup> The influence of adrenal and neurally mediated catecholamine release on blood pressure have also been studied in human pregnancy, but the data are often contradictory and difficult to interpret.<sup>2-13</sup> Reasons for discrepant observations may be technical factors including blood sampling under poorly controlled conditions and a variety of problems related to the catecholamine assay, but the major criticisms of most reports relate to protocol design. Many investigators studied their subjects during basal conditions only, often obtained but a single sample, and failed to restudy the same women post partum.<sup>2-7</sup> In

addition, there are only a few investigations<sup>8, 10-13</sup> that have focused on the role of catecholamines during dynamic blood pressure regulation, that is, changes occurring during physiologic stresses such as assuming an upright posture or exercise; these are the crucial studies that must be performed if we are to adequately define the physiology of the adrenergic system during normal and abnormal gestation.

The present protocol, designed to investigate catecholamine release under a variety of physiologic maneuvers, focuses on comparison of measurements made during and after gestation in the same subjects. Each woman was studied under basal conditions, after changing from a lateral recumbent to a supine position, after standing, and finally during isometric exercise. During some of the maneuvers we also monitored the renin-aldosterone system, since it too is involved in blood pressure control.

## Methods

Studies were performed during the third trimester and again at least 6 weeks post partum on 13 black subjects (12 primigravid and 1 secundigravid), ages 15 to 19, recruited from the antenatal clinic at the University of Chicago Lying-in Hospital. The research protocol was approved by the institutional clinical investigation committee, and informed consent was obtained in writing from each subject. All volunteers were free of cardiovascular, hematologic, and renal disease as determined by routine history, physical examination, and

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**Table 1.** Physiologic responses to postural maneuvers and isometric exercise during and after pregnancy

	<i>Lateral decubitus position</i>		<i>Supine recumbency</i>		<i>Standing</i>	<i>Handgrip</i>
	<i>Measurement 1</i>	<i>Measurement 2</i>	<i>5 min</i>	<i>10 min</i>		
Systolic blood pressure (mm Hg)						
Pregnant	104 ± 3	106 ± 3	113 ± 5	109 ± 5	119 ± 4	137 ± 3
Postpartum	107 ± 3	106 ± 2	117 ± 3	117 ± 3	122 ± 2	140 ± 4
Diastolic blood pressure (mm Hg)						
Pregnant	56 ± 1	56 ± 2	63 ± 3	63 ± 3	70 ± 3	89 ± 4
Postpartum	54 ± 2	55 ± 2	65 ± 2	64 ± 2	78 ± 2	93 ± 5
Heart rate (bpm)						
Pregnant	79 ± 2*	80 ± 3*	90 ± 5*	89 ± 5*	100 ± 2	121 ± 4
Postpartum	61 ± 2	61 ± 2	61 ± 2	60 ± 2	100 ± 3	118 ± 6
Norepinephrine (pg/ml)						
Pregnant	125 ± 22	126 ± 23	148 ± 31	131 ± 21	252 ± 29	286 ± 36
Postpartum	117 ± 9	128 ± 11	109 ± 9	126 ± 10	377 ± 40†	520 ± 47†
Epinephrine (pg/ml)						
Pregnant	51 ± 9	40 ± 7	84 ± 49	34 ± 4	66 ± 9	82 ± 23
Postpartum	39 ± 5	42 ± 6	35 ± 4	34 ± 4	56 ± 8	91 ± 13
Plasma renin activity (ng/ml/hr)						
Pregnant	6.2 ± 0.4*	6.4 ± 0.5*	6.3 ± 0.4*	6.5 ± 0.4*	7.7 ± 0.6*	8.7 ± 0.9*
Postpartum	1.9 ± 0.5	1.7 ± 0.5	1.4 ± 0.4	1.5 ± 0.4	2.5 ± 0.7	3.5 ± 0.9
Aldosterone (ng/dl)						
Pregnant	39.1 ± 6.5*	38.2 ± 5.9*	34.7 ± 3.8*	36.3 ± 7.5*	50.3 ± 9.8*	—
Postpartum	3.7 ± 1.5	7.4 ± 1.5	7.0 ± 1.5	6.4 ± 0.8	9.0 ± 2.5	—

Mean ± SEM, n = 11 to 13 in each group.

\*p < 0.001 versus postpartum levels.

†See text for discussion of significant differences.

laboratory tests, and all remained normotensive during and following pregnancy. Medications included only iron and vitamins during the antenatal period studies. All infants were delivered at or beyond the thirty-seventh gestational week and were of size appropriate for gestational age. After delivery, three subjects were taking oral contraceptives; however, analysis of the data failed to reveal significant differences between these subjects and those not taking oral contraceptives, and therefore the results from all subjects have been combined.

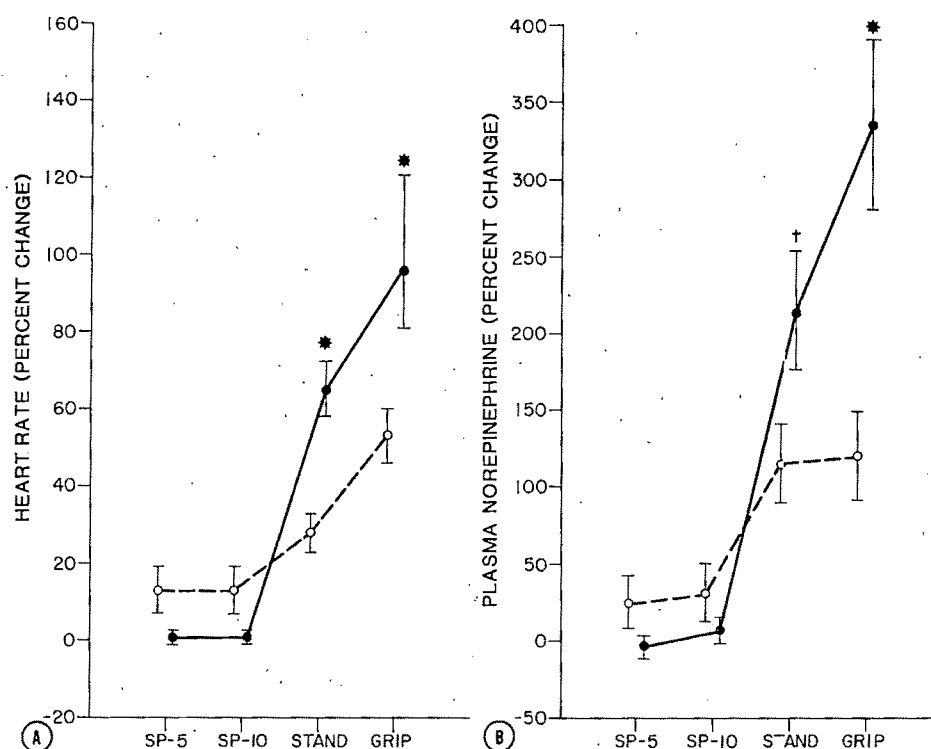
Subjects were admitted to the Clinical Research Center 24 hours before the initiation of the protocol, during which time activity was unrestricted and a regular diet was consumed. Caffeine-containing beverages and tobacco were withheld for at least 12 hours before each study. Between 7 and 8 AM, with the volunteer in the fasting state, a 19-gauge catheter for bloodletting was placed in an arm vein and attached to a syringe containing heparinized saline solution (100 U/ml) to maintain catheter patency. The subject was then placed in the lateral recumbent position, and a blood pressure cuff, placed on the uppermost arm, was attached to an automatic recording device (Arteriosonde 1216, Roche Medical Electronics, Cranbury, New Jersey, or Dinamap Model 845XT, Critikon Inc., Tampa, Florida). Pulse rate was determined by standard electrocardiography or the Dinamap physiologic recorder. After a period of at least 30 minutes, during which time blood

pressure and pulse were observed to be stable, arterial pressure and pulse were recorded and blood was sampled at specific intervals for plasma levels of norepinephrine, epinephrine, renin activity, and aldosterone in the following sequence: (1) after lateral recumbency—two determinations 5 minutes apart; (2) after 5 and 10 minutes of supine recumbency; (3) after 10 minutes of standing; and (4) after 2 minutes of 50% maximal handgrip exercise using a rolled blood pressure cuff or dynamometer (maximal grip was determined before the equilibration period).

Blood was collected in chilled heparinized tubes for catecholamines and aldosterone and in ethylenediaminetetraacetate-containing tubes for plasma renin activity. Samples were kept on ice and centrifuged at 4° C immediately following each study, and plasma was stored at -20° C until assay. Levels of epinephrine and norepinephrine were determined by radioenzymatic assay,<sup>14</sup> plasma renin activity by radioimmunoassay measurement of generated angiotension I, and aldosterone by radioimmunoassay.

To maintain a balanced statistical analysis, responses for each subject were included only for those maneuvers completed both during and following gestation. Comparison of parameters in the basal state were performed with paired two-tailed Student's *t* test. Comparisons of the changes in each parameter during and after pregnancy were performed using analysis of variance and two-tailed Student's *t* tests. To adjust for mul-





**Fig. 1.** A, Percent change (mean  $\pm$  SEM) in heart rate in response to 5 and 10 minutes of supine recumbency (SP-5 and SP-10, respectively), 8 minutes of quiet standing, and 2 minutes of 50% maximal handgrip. Basal values were calculated as the mean of two determinations during lateral recumbency shown in Table I. \* $p < 0.01$  compared to pregnant level. B, Percent change (mean  $\pm$  SEM) in plasma norepinephrine concentrations in response to 5 and 10 minutes of supine recumbency, standing, and handgrip. Basal value calculations, abbreviations, and probabilities as defined in A. †The increase after standing was greater in the postpartum studies ( $214 \pm 128$  compared to  $116 \pm 83$ ), but this difference was significant only at the  $p < 0.05$  level.

tiple comparisons, only  $p$  values  $< 0.01$  were considered significant. Data are presented as mean  $\pm$  SEM.

### Results

Each protocol was initiated after the subject had attained a stable basal state as indicated by the constancy of serially measured hemodynamic and hormonal parameters during lateral recumbency (Table I). Under these conditions systolic and diastolic blood pressure, as well as plasma levels of epinephrine and norepinephrine, were similar in pregnant and postpartum subjects. In contrast, pulse rate was significantly ( $p < 0.001$ ) greater during gestation. When turned from lateral to supine recumbency, pulse rate and plasma norepinephrine tended to increase in the pregnant subjects (although these changes were not significant), whereas after delivery no such trend was observed. It is of note that in one pregnant subject mean arterial pressure fell 26 mm Hg in the supine position, accompanied by twofold and fivefold increases in plasma levels of norepinephrine and epinephrine, respectively. When she returned to the lateral decubitus position, blood pressure rapidly normalized.

Following 8 minutes of standing, blood pressure and pulse were similar in pregnant and postpartum subjects. However, since the former had a significantly greater pulse rate in the supine position before standing, the increment in heart rate in response to upright posture was significantly ( $p < 0.001$ ) greater in the non-pregnant subjects. This was accompanied in the postpartum studies by higher levels of plasma norepinephrine at the end of the upright period and a significantly ( $p < 0.01$ ) greater increase in these levels when supine and standing positions were compared. Similar changes were observed when the percentage increase from basal, lateral recumbent values were compared (Fig. 1). In contrast, the increment in plasma epinephrine in response to standing was similar during and after pregnancy.

After 2 minutes of isometric exercise, blood pressure, pulse, and plasma epinephrine levels were similar in pregnant and postpartum subjects. Although the increase in plasma norepinephrine levels above those observed after quiet standing was not significant in either group, the mean increment in the postpartum women ( $143$  pg/ml) tended to be greater than that in pregnant

subjects (34 pg/ml). However, when the difference between basal (lateral recumbent) and postexercise norepinephrine levels and heart rate were compared, the increment in each was significantly less during gestation (Fig. 1). Plasma renin activity and aldosterone were significantly greater ( $p < 0.001$ ) in the pregnant subjects during all maneuvers. In contrast to results for plasma norepinephrine, increments in both plasma renin activity and aldosterone in response to upright posture and exercise were similar during and following gestation.

### Comment

We studied adrenergic function in pregnancy using a carefully controlled environment made possible within a clinical research center. The data demonstrate that basal plasma catecholamine levels are similar during and after gestation, but responses to physiologic stress such as upright posture and exercise are significantly altered in late gestation.

While most authors have reported that basal plasma and 24-hour urinary catecholamines are similar in pregnant and nonpregnant subjects,<sup>2,5, 7-9</sup> others have described differences.<sup>6, 11, 12</sup> Natrajan et al.<sup>6</sup> observed a significant decrease in plasma epinephrine and norepinephrine concentrations as pregnancy progressed, and Nisell et al.<sup>11</sup> reported significantly lower arterial plasma epinephrine levels during gestation. In contrast, Whittaker et al.<sup>12</sup> noted that basal venous plasma levels of both catecholamines were increased during pregnancy. Consideration of these discrepant findings requires a review of the many pitfalls inherent in catecholamine research.

Interpretation of plasma catechol levels is complicated by methodologic difficulties in sample collection and measurement, as well as the complexities of catecholamine metabolism.<sup>1, 15, 16</sup> Because of the lability of plasma norepinephrine and epinephrine levels, strict attention to environmental circumstances, time factors, and drugs used (including caffeine and nicotine) is required. The manner in which the blood is sampled is also important as even the slight stress of venipuncture may elevate circulation catecholamines substantially.<sup>15</sup> Similar considerations dictate that the best protocols comparing pregnant and nonpregnant populations are those in which the same individuals are tested serially.

With the above considerations in mind, the present studies were performed in a clinical research center where environmental circumstances could be controlled more carefully and duplicated during antenatal and postpartum tests. Each protocol was initiated during the same hour of the day, and the subjects, positioned in lateral recumbency, were allowed an adequate equilibration period after placement of the intravenous catheter. The humoral and hemodynamic values re-

corded during two successive control periods (Table I) indicate that subjects were indeed in a resting steady state. Use of lateral recumbency for basal collections was felt to be important as aortocaval compression may have significant effects (for as noted in the results section, one pregnant subject who changed to a supine position developed marked hypotension associated with tachycardia and elevation of plasma catecholamines, all abnormalities reverting quickly on resumption of lateral recumbency). The success of this approach seems to be validated by the observation that humoral and hemodynamic parameters recorded during two successive control period were stable, and we feel strongly that the similar plasma norepinephrine and epinephrine levels during and after pregnancy accurately represent conditions in the basal state.

Observation of similar catecholamine levels in pregnant and nonpregnant populations has led to the suggestion that sympathoadrenal function is not significantly altered during gestation. Such conclusions were obviously premature, given the paucity of information concerning the catecholamine response of pregnant women during maneuvers that involve dynamic blood pressure regulation. Although Rubin et al.<sup>8</sup> observed similar increments in plasma norepinephrine in women standing for 5 minutes during the third trimester and on the fifth day of the puerperium, two recent, more complete investigations suggest a contrary view. Nisell et al.<sup>10</sup> and Whittaker et al.<sup>12</sup> reported that heart rate and plasma catecholamines increased less in response to standing or upright tilt in normal pregnant women compared to nonpregnant control subjects. Our results also suggest a blunted noradrenergic response in late pregnancy, for after 8 minutes of quiet standing plasma norepinephrine increments were less during than after gestation and, like Nisell et al., we too noted attenuation of the anticipated rise in heart rate when pregnant subjects stood (Table I and Fig. 1).

In contrast to the findings of Nisell et al.<sup>10</sup> and Whittaker et al.,<sup>12</sup> we observed similar increases in plasma epinephrine levels when the women stood during or after pregnancy. Their observations are, however, more consistent with the attenuated rise in heart rate when pregnant subjects stood, and we have no explanation for this discrepancy.

We also noted a decreased plasma norepinephrine response in pregnant women undergoing isometric exercise; the differences in test results during and after gestation, when viewed as the percent increase from basal values, were even more marked than when the effects of upright posture were compared (Fig. 1). Nisell et al.<sup>11</sup> also studied the effects of isometric exercise in pregnancy; however, they noted similar increments in heart rate and plasma norepinephrine levels in pregnant and postpartum women. The discrepancy might

reflect protocol design, particularly the fact that they measured arterial whereas we determined venous plasma levels and that the latter reflect catecholamines produced by sympathetic activity within the forearm.<sup>16</sup> Arterial sampling was precluded in our study of normal volunteers so that this explanation must remain speculative. In the only other investigation of exercise we could locate, Rabinovici et al.<sup>13</sup> also noted a blunted norepinephrine response to isometric exercise in pregnant women undergoing spontaneous labor when compared to nonpregnant women.

Finally, we took advantage of this study to simultaneously monitor the renin-angiotensin-aldosterone system during the various postural and exercise protocols. Plasma renin activity and aldosterone increased, albeit only minimally, and in contrast to differences in adrenergic responses during pregnancy, alterations in the renin-angiotensin system after provocative maneuvers were similar during and after gestation.

In conclusion, our data suggest that pregnancy alters the response of the sympathetic nervous system to upright posture and isometric exercise in that increments in heart rate and norepinephrine levels are attenuated in gestation. These results agree in part with Nisell et al.,<sup>10</sup> who also noted attenuated responses in pregnant women during tilting, and with Whittaker et al.<sup>12</sup> and Rabinovici et al.,<sup>13</sup> who observed blunted plasma norepinephrine responses after standing and isometric exercise during gestation. These conclusions are made cautiously as they are based on the assumption that changes in circulating catecholamine levels are accurate indices of sympathetic nervous system responsiveness during dynamic testing. Such conclusions can only be validated or rejected when more is known about neuronal uptake and peripheral metabolism of catecholamines both during and after pregnancy.

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# Kell sensitization in pregnancy

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Maternal anti-Kell antibody was found in 127 of 127,076 pregnancies during a 16-year period (0.1%). Thirteen Kell-sensitized pregnancies ended with a Kell-positive newborn infant, five of these had a poor perinatal outcome (hydrops, intrauterine or neonatal death, hemoglobin <7.9 gm, congestive heart failure). Mothers with Kell-positive infants and poor outcome had anti-Kell titers  $\geq 1:128$  at delivery. With a maternal anti-Kell titer <1:32 at delivery, only one baby was Kell positive and mildly affected by hemolytic disease. Spectrophotometric analysis of amniotic fluid (delta optical density at 450 nm) in three of four pregnancies with poor perinatal outcomes revealed values of delta optical density at 450 nm in the high midzone of Liley within 1 week of delivery. Therefore, Kell-sensitized patients have to be managed differently from patients with rhesus sensitization. A management scheme to optimize perinatal outcome in Kell-sensitized pregnancy is described on the basis of this largest reported series of Kell-sensitized pregnancies. (AM J OBSTET GYNECOL 1986;154:85-90.)

**Key words:** Kell sensitization, Kell isoimmunization, irregular antibodies, erythroblastosis fetalis, hemolytic disease of the newborn

Hemolytic disease of the newborn is most commonly due to Rh isoimmunization.<sup>1</sup> Since the introduction of anti-D immunoglobulin (RhoGAM) in 1968, the incidence of Rh isoimmunization has been dramatically reduced. Obstetricians are now increasingly aware that irregular antibodies can cause hemolytic disease of the newborn. The irregular antibodies are non-Rh antibodies such as Kell, Kidd, Duffy, and others. Antibodies produced by the maternal immune systems to these blood group antigens are G immunoglobins and are able to cross the placenta and affect the fetus. Irregular antibodies are increasing in pregnant women because of two factors: the decrease in Rh disease and thus a relative increase in irregular antibodies and an increased number of blood transfusions in obstetric practice causing sensitization. The irregular antibody that is thought to be the most potent and most commonly associated with hemolytic disease of the newborn is the Kell antibody.<sup>1</sup> Information on Kell isoimmunization in pregnancy is based on case reports.<sup>2-11</sup> Presently, management of Kell isoimmunization is generally the same as that for rhesus isoimmunization. However, experience at our institution as well as two case reports<sup>10, 11</sup> of Kell isoimmunization suggest that the use of the criteria for the management of rhesus isoimmunization may not be appropriate for Kell isoimmunization.

We reviewed the pregnancy course and outcome of Kell-sensitized patients managed during the past 16 years at our institution. Our purpose was to determine

the factors that best predict fetal and neonatal outcome in patients with Kell isoimmunization.

## Methods

Records of all Kell-sensitized obstetric patients at Magee-Womens Hospital from 1969 to 1984 were reviewed. The patients were divided into those who had abortions and those who had been delivered of children. Records of all viable babies were examined. If the baby had a positive direct antiglobulin (Coombs) test and the eluate contained Kell antibodies that reacted with Kell-positive cells on the standard blood bank screening panel, then the baby was considered to be Kell positive and the sensitization due to Kell antibody. We also included Kell-positive mothers who had other antibodies such as D. If the mother was not delivered of her infant at Magee-Womens Hospital, we attempted to trace her records.

The maternal chart was reviewed for gravidity, parity, gestational age, prior blood transfusion, past obstetric history, and maternal blood type. Antibody titer was examined at the first prenatal visit and at delivery. If available, the genotype of the father of the baby was also evaluated. Finally, the results of spectrophotometric analysis of amniotic fluid for bilirubin and ultrasound examinations were reviewed.

Neonatal charts were reviewed for weight, sex, and Apgar scores. The results of the direct antiglobulin (Coombs) test, eluate on fetal cells, cord hemoglobin, reticulocyte count, and cord bilirubin were recorded. Peak bilirubin concentrations, exchange transfusions, and phototherapy were also reviewed. Evidence for hepatosplenomegaly, effusions, edema, or hydrops in the infants was examined. When necessary, autopsy records were reviewed.

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**Table I.** Clinical data on pregnancies with Kell-positive babies

Patient	Gravidity and parity	Paternal genotype	Blood transfusion	Obstetric history	Anti-K		$\Delta OD_{450}$ (last)	Sonar (last)	Gestational age at delivery	Apgar score	
					Screen	Delivery				1 min	5 min
A. H. No. 1	3-0	Kk	Yes	NL	1:512	1:256	0.262	H,P	27	3	2
A. H. No. 2	4-1	Kk	Yes	P-NND	1:64	1:128	0.195	A	25	0	0
M. J.	2-1	—	No	NL	+	1:128	0.135	H,P	31	2	8
M. S.	3-1	KK	No	NL	1:128	1:128	0.018	NL	36	9	9
T. B.	1-0	—	Yes	NL	1:128	1:256	0.083*	NL	33	7	8
C. W.	3-2	—	Yes	NL	?	1:128	—	—	39	4	7
M. B.	4-3	—	Yes	MS	1:256	1:256	0.032	—	36	8	9
					D1:32	D1:256					
C. P.	3-2	—	Yes	NL	+	1:256	—	—	42	9	—
G. K.	6-3	—	Yes	NL	—	1:32	—	—	39	9	9
P. B.	2-1	—	Yes	NL	1:32	1:64	0.010	IUGR	39	9	9
					D1:32	D1:128	(2 wk PTD)				
J. C.	2-1	—	Yes	NL	neg	1:8	—	—	39	9	9
A. S.	2-1	K+	No	NL	1:256	1:512	0.015	NL	38	4	7
D. K.	2-1	K+	No	NL	?	1:4096	—	IUFD	21	0	0

A = ascites; CHF = congestive heart failure; EL = eluate on fetal blood; H = hydrops; IUFD = intrauterine fetal death; IUGR = intrauterine fetal growth retardation; MS = mild sensitization; NA = not applicable; NL = normal; NND = neonatal death; P = polyhydramnios; PTD = prior to delivery; S = survivor.

\*Blood in amniotic fluid; tap 1½ weeks before delivery.

In one case the mother was delivered at a referral hospital and was included because telephone consultation with the authors had been obtained. One patient had two Kell-sensitized pregnancies.

We considered a poor outcome to be any of the three following findings: hydrops, cord hemoglobin <7.9 gm, or perinatal death. Hydrops was defined as generalized edema and ascites.

### Results

Among 127,076 patients delivered during the 16-year period from 1969 to 1984, 120 patients had 127 pregnancies with Kell antibody present in the maternal serum. Of these 127 pregnancies, 13 had Kell-positive babies or anti-Kell antibody on the fetal red blood cells. Sixty-nine babies were Kell negative; however, five of these babies were also Rh sensitized and had a positive direct antiglobulin (Coombs) test secondary to anti-D only. Twenty-nine babies had no evaluation at delivery despite the mother's sensitization to Kell. Review of these 29 patients revealed that none of the children needed phototherapy or exchange transfusion. Ten pregnancies ended in therapeutic abortion and five in spontaneous abortion. There were no sets of twins. One patient's chart could not be found. In eighteen of the 127 pregnancies, multiple antibodies were found in the maternal serum. The most common combinations were K, D, and K, c, of which there were five each. There were three with K, D, C, and five combinations of K with other irregular antibodies. Two babies of mothers with multiple antibodies had red blood cell eluates that were positive for K and D.

Thirteen of the 127 Kell-sensitized pregnancies re-

sulted in an affected newborn infant. Five of these 13 Kell-sensitized pregnancies had a poor outcome. All 13 pregnancies were evaluated in detail to determine the predictive value of the maternal, fetal, and neonatal parameters and to delineate a scheme to manage the Kell-sensitized pregnancy.

The 13 Kell-positive babies were delivered by 12 mothers. These mothers ranged in age from 18 to 36 years. Ten patients were multiparous and two were nulliparous. The blood types were as follows: six were A+, three were O+, one was B+, and two were O-. Paternal Kell genotype was examined only four times. Eight of the 12 Kell-sensitized patients had blood transfusions. All patients with a good outcome had a prior blood transfusion, while only one of four with a poor outcome had a prior blood transfusion.

The obstetric histories of the patients with Kell-positive neonates were investigated. One patient, A. H., had two poor pregnancy outcomes. After a spontaneous abortion at 18 weeks, this patient had a neonatal death in her second pregnancy that was due to fetal hydrops resulting from Kell antibody. Her third pregnancy ended in a hydropic stillborn infant at 25 weeks' gestation. The other three patients with poor outcomes had normal past obstetric histories. The eight patients with good outcomes had no prior history of Kell-sensitized babies or problems with prior pregnancies.

Prenatal antibody screening results were available in only 10 of 13 pregnancies in which a Kell-positive baby was eventually delivered. Of the five cases with poor outcomes, one titer was not determined, one titer was reported as positive only, and the other three titers were 1:64, 1:256, and 1:512. At birth, a titer was available

Birth weight (gm)	Direct Coombs	Bilirubin		Cord hemoglobin	Hepatomegaly/splenomegaly	Photo-therapy	Exchange transfusion	Outcome	EL
		Cord	Peak						
1220	+3	2.4	—	2.1	++/++	—	No	H, NND	+
370	—	—	—	—	++ (Sonar)	—	No	H, IUFD	NA
1760	+3	2.6	8.4	7.5	++/++	+	No	H, S	+
2720	+3	2.5	4.5	13.3	—/—	—	No		+
2420	+3	3.7	12.0	14.6	—/—	+	No		+
3270	+3	10.5	17.1	12.6	++/++	+	×1		+
2940	+4	3.4	16.0	11.6	—/++	+	×1		+ K + D
4240	+1	—	—	—	—/—	—	No		+
3360	+3	3.0	4.8	17.0	+/—	—	No		+
2300	+4	3.6	15.1	20.0	—/—	+	No		+ K + D
3180	+2	2.5	—	14.5	—/—	—	No		+
2960	+4	1.0	5.8	3.5	++/++	+	×1	CHF	+
615	—	—	—	—	++/++	—	No	H, IUFD	NA

in all pregnancies (Table I). In five pregnancies resulting in a poor outcome, the anti-K titers at delivery ranged from 1:128 to 1:4096. In the eight pregnancies with good outcome, the anti-K titers at delivery ranged from 1:8 to 1:256.

The bilirubin concentration in amniotic fluid, a measure of fetal hemolysis and anemia, was determined spectrophotometrically as the difference between expected and actual values at a wavelength of 450 nm (delta optical density at 450 nm,  $\Delta OD_{450}$ ). These values were plotted on the graph developed by Liley for patients with Rh isoimmunization.<sup>12</sup> Determinations of the  $\Delta OD_{450}$  value were performed in eight of the 13 pregnancies with Kell-positive babies. In six cases, the  $\Delta OD_{450}$  value was measured within 1 week of delivery. Three of the five pregnancies with poor outcomes had  $\Delta OD_{450}$  values in the high midzone according to Liley<sup>12</sup> (Fig. 1). It is of special interest that none of these values was in the high zone, which is most predictive of poor outcome in rhesus-sensitized patients. In one patient with poor outcome, amniotic fluid analysis was not done. There was only one patient with a  $\Delta OD_{450}$  value in the high midzone who had a good outcome; however, this patient had a bloody tap performed 1½ weeks before delivery. There were two other patients who had  $\Delta OD_{450}$  values in the high midzone but these fell into the low midzone or low zone at subsequent amniocenteses. The  $\Delta OD_{450}$  values of these two patients within 1 week of delivery were in the low midzone or low zone. Patient A. S. deserves special comment. Her pregnancy was evaluated with serial amniocenteses from 28 weeks through delivery at 37 weeks. The  $\Delta OD_{450}$  values fell progressively from the high midzone into the low zone. At delivery, her baby developed fetal distress and emergency cesarean section was performed. It was found that the baby had a hemoglobin

of 3.5 gm secondary to Kell isoimmunization and subsequently developed congestive heart failure. Cardiac catheterization results in this infant were normal.

Eight of the 13 Kell-positive babies had prenatal sonar evaluation including all five cases with poor outcome. Four babies had ascites or hydrops. The sonar results in the fifth patient (A. S.) with poor outcome appeared normal 4 days before delivery. Congestive heart failure developed shortly after delivery in the absence of ascites or hydrops; however, a pericardial effusion was present. Sonar examination in Patient A. H. at 24 weeks' gestation revealed fetal ascites. The  $\Delta OD_{450}$  value at that time was in the high midzone. She was told to return the next day for further evaluation and treatment; however, she returned 1 week later with a hydropic dead fetus. All babies alive before delivery and with poor outcomes were delivered by cesarean section because of hydrops or fetal distress.

The direct antiglobulin (Coombs) test was evaluated in each baby. All babies born alive with poor outcomes had 3+ or 4+ antiglobulin (Coombs) test results. The direct antiglobulin (Coombs) test did not correlate with cord hemoglobin or serum bilirubin concentration.

Hemoglobin values in umbilical cord blood obtained in 10 of 11 live-born infants ranged from 2.1 to 20 gm. Infants with hemoglobin values of <7.9 gm had either hydrops or congestive heart failure. All infants with a hemoglobin >7.9 gm at birth had neither of these findings. A hemoglobin concentration <13 gm was always associated with moderate to severe hepatosplenomegaly. Too few reticulocyte counts were performed to be of any predictive value.

Five of the 11 live-born Kell-positive infants required no special treatment. Six infants needed phototherapy and three of these needed one exchange transfusion in addition to control hyperbilirubinemia (Patients



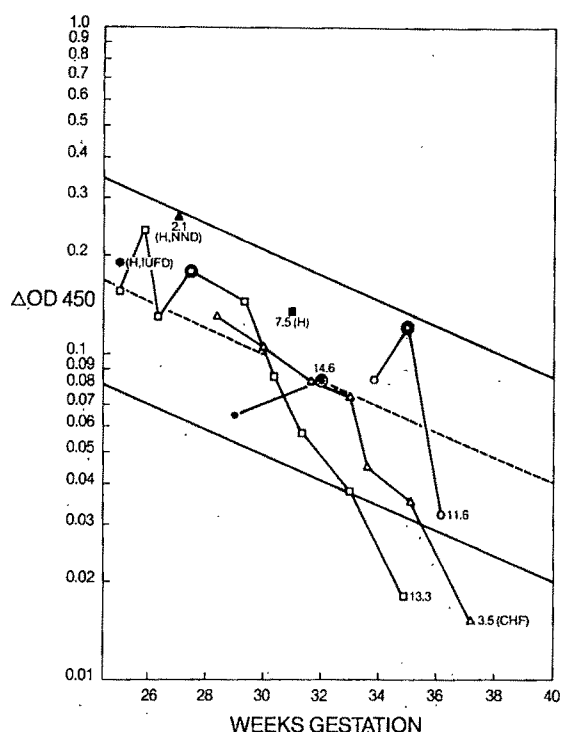


Fig. 1.  $\Delta OD_{450}$  values compared to gestational age in seven patients who were delivered within 1 week of the last  $\Delta OD_{450}$  determination. Numbers indicate hemoglobin values in umbilical cord blood. H = Hydrops; IJFD = intrauterine fetal death; NND = neonatal death; CHF = congestive heart failure. Encircled values indicate blood-tinged amniotic fluid. Neonate delivered with hemoglobin of 14.6 gm had amniocentesis 1½ weeks before delivery.

A. S., M. B., and C. W.). The newborn infant in the first pregnancy of Patient A. H. died 30 minutes after delivery before any such therapy could be instituted.

### Comment

Antibodies are present in 3.1% to 5.7% of pregnant women.<sup>13,14</sup> Of all the identified antibodies, 5.3% to 38% have been reported to be irregular antibodies.<sup>1,13,14</sup> In several series of antibody investigations in pregnant women, Kell was found to be the most common irregular antibody outside of the Rh blood group system,<sup>1,13,14</sup> capable of causing fetal erythroblastosis.

In the present series of 127,076 deliveries, we found a positive anti-Kell screen in 127 pregnancies (0.1%). Quénan et al.<sup>13</sup> and Polesky<sup>14</sup> found Kell antibody in 0.16% and 0.21% in series of 18,378 and 43,000 patients, respectively.

The purpose of our study was to delineate predictive factors for the management of the Kell-sensitized patient. Past obstetric history in the 11 multiparous patients with Kell-positive babies revealed only one patient (A. H.) with a previous poor pregnancy outcome. In Rh-negative patients, it is calculated that if the baby in a prior pregnancy is sensitized but normal, the

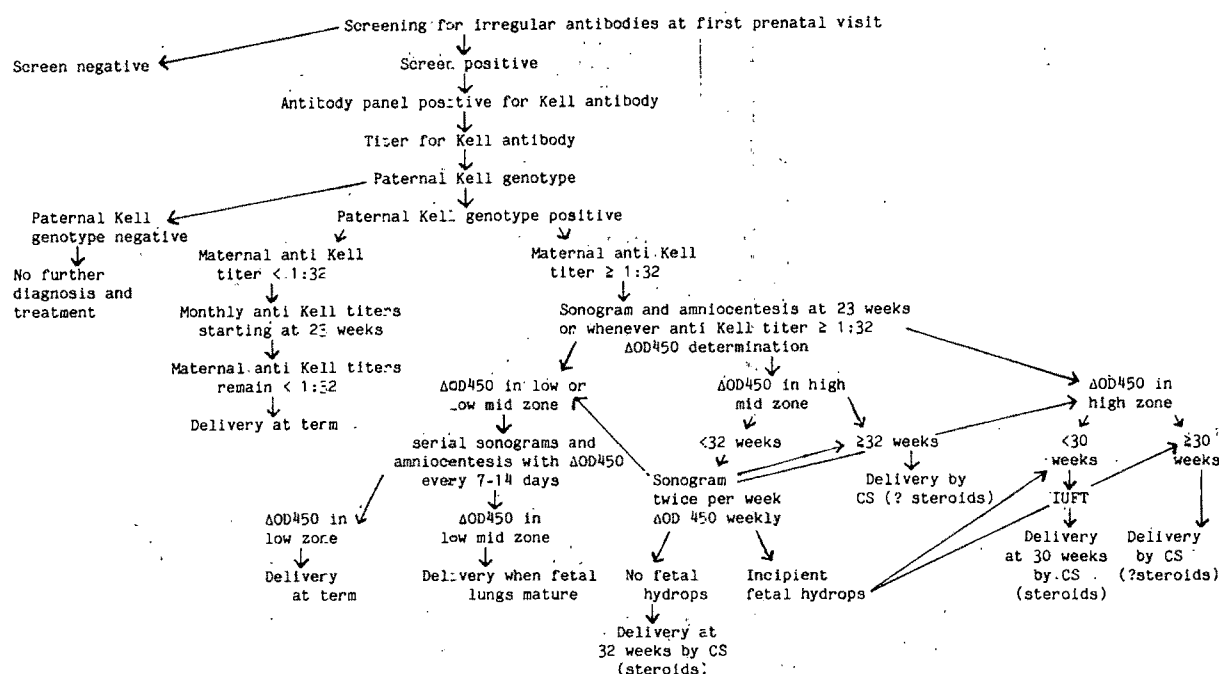
chance of a stillbirth is 2%.<sup>15</sup> In contrast, three of five pregnancies with poor outcome in our study had histories that gave no indication that the patient would have a seriously affected infant. Thus obstetric history appears less predictive in Kell-sensitized pregnancy, unless the patient's prior outcome is extremely poor.

Since many patients are sensitized to irregular antibody by blood transfusion, we investigated whether the titer at delivery could predict a Kell-positive or Kell-negative baby. At a titer of  $<1:32$ , one baby was Kell positive (2.6%) and 38 babies were Kell negative. When the titer was  $\geq 1:32$ , 12 babies were Kell positive (28.6%) and 30 babies were Kell negative. No babies with a Kell titer of  $<1:32$  died in our series or in the case reports in the literature.<sup>2-11</sup> There are two cases, one in our series and one in a report by Spivey et al.,<sup>9</sup> with a titer of  $<1:32$  and a positive direct antiglobulin (Coombs) test in the newborn infant; however, both cases of hemolytic disease were mild. All mothers with babies that had a poor outcome in our series had titers  $\geq 1:128$  at delivery.

Prenatal screening for irregular antibodies is essential, together with the knowledge that Kell antibody can cause hemolytic disease in the fetus. In one patient, M. J., fetal hydrops was discovered by sonar done for polyhydramnios at 32 weeks' gestation. Upon review of her antenatal chart, it was realized that an anti-Kell antibody at the time of screening at the first prenatal visit had been ignored. Rescreening for atypical antibodies at 28 weeks in Rh-positive patients has been suggested but not officially recommended by the American College of Obstetricians and Gynecologists. This practice may help in the management of patients with irregular antibodies; however, the cost effectiveness of this approach is doubtful. Genotyping of the father of the baby for Kell zygosity is important in the management of pregnant women with Kell sensitization, but in only four of our 13 pregnancies with Kell-positive babies was a paternal Kell genotype performed. As 91% of the population is Kell negative, amniocentesis might be obviated in patients in whom the baby's father is Kell negative.

Blood transfusion is a common method of sensitization to irregular antibodies. Eight of the 12 Kell-sensitized patients with Kell-positive babies had prior blood transfusion in our series (67%). To avoid pregnancies with Kell sensitization due to blood transfusion, Pepperell et al.<sup>16</sup> suggested that women below the age of 50 be transfused with Kell-negative blood. This is currently done in Australia.

The mode of sensitization appears to be important for pregnancy outcome. Of our four patients with a poor outcome, three had no blood transfusions, and one patient (A. H.) with two pregnancies had a history of blood transfusion and prior pregnancies. It is prob-



**Fig. 2.** Management of Kell-sensitized pregnancies. (steroids) = After maternal steroid administration to accelerate fetal lung maturity. (? steroids) = After maternal steroid administration if fetal lung profile was immature. CS = Cesarean section. IUFT = Intrauterine fetal transfusion.

able that all patients in our series were sensitized by a prior pregnancy or a combination of pregnancy and blood transfusion (A. H. had a Kell-positive husband). Pepperell et al.<sup>16</sup> reported similar findings in their study.

The keynote of management of Rh disease is spectrophotometric analysis of amniotic fluid and determination of the  $\Delta OD_{450}$  value. In 1961, Liley<sup>12</sup> discovered that by determining the  $\Delta OD_{450}$  value in amniotic fluid within 1 week of delivery, one could predict rather accurately the hemoglobin level of the baby at delivery. Our series as well as two case reports<sup>10, 11</sup> suggests that in Kell-sensitized patients, compared with Rh-sensitized patients, more serious hemolytic disease develops in the fetus at lower  $\Delta OD_{450}$  values. In our series of pregnancies with poor outcomes, three of four pregnancies with  $\Delta OD_{450}$  determinations had values in the high midzone within 1 week of delivery. No fetus had a  $\Delta OD_{450}$  value in the high zone of Liley. Berkowitz et al.<sup>11</sup> described a Kell-sensitized patient who had a  $\Delta OD_{450}$  value in the high midzone 2 weeks before a fetal death with hydrops at 24 weeks. Birkenfeld et al.<sup>10</sup> described a case similar to A. S. in our series. The  $\Delta OD_{450}$  value before cesarean section in this patient was 0.06 (low midzone) at 31 weeks' gestation and the fetus had a hemoglobin of 2.0 gm but no hydrops. They ascribed the anemia to Kell isoimmunization.

Fetal hydrops was present in three of five pregnancies in which the initial  $\Delta OD_{450}$  value was in the high midzone. Two patients (M. S. and M. B.) had decreases in the  $\Delta OD_{450}$  value from the high midzone into the low

zone or low midzone with normal outcomes. Thus, a Kell-sensitized patient with a  $\Delta OD_{450}$  value in the high midzone is at much higher risk of fetal hydrops than an Rh-sensitized patient.

Sonar has been used increasingly in recent years to help manage patients with hemolytic disease of the fetus. Six patients in our series were evaluated by sonar. Two hydropic infants and three normal infants were accurately identified. In one patient (A. S.) no hydrops was noted in the infant. Pericardial effusion present at birth may have developed in the 4 days between the time of the sonar and the time of delivery of the infant.

Kell antigenic potency, or the ability of the maternal immune system to produce Kell antibody, is reported to be the highest of all irregular antibodies. Potency of the Kell antibody in causing hemolytic disease of the newborn is uncertain. In our series, 38% of the Kell-positive babies had severe hemolytic disease.

On the basis of the results of our study, which represents the largest reported series of Kell-sensitized pregnancies, and the case reports in the literature,<sup>2-11</sup> we propose the management scheme for Kell-sensitized pregnancies depicted in Fig. 2. Paternal Kell genotyping in the presence of maternal anti-Kell antibody allows exclusion of 91% of cases in which the fetus will be Kell negative, thus requiring no further diagnosis or treatment. Maternal anti-Kell titer determinations allow identification of patients who require amniocentesis and  $\Delta OD_{450}$  determinations. The  $\Delta OD_{450}$  values in the high midzone require intensive follow-up to identify

incipient fetal hydrops. This approach is distinctly different from the management of Rh-hemolytic disease. The high risk of fetal hydrops and perinatal death with  $\Delta OD_{450}$  values in the high midzone in patients with Kell sensitization necessitates this intensive surveillance with sonograms twice per week and  $\Delta OD_{450}$  determinations weekly. Intrauterine fetal transfusion and/or timed preterm delivery may be indicated after maternal steroid administration to accelerate fetal lung maturation. Fetal hydrops with  $\Delta OD_{450}$  readings in the high midzone frequently seen in Kell-sensitized pregnancy is very rarely seen in rhesus-sensitized pregnancy. This difference may be due to different degrees of hypoproteinemia. With extremely high maternal anti-Kell titers, intrauterine fetal death due to hydrops may occur before 23 weeks' gestation (Patient D. K.). Occasionally severe degrees of fetal anemia may develop even though  $\Delta OD_{450}$  values have been in the low zone (Patient A. S.). Nevertheless, the proposed management scheme optimizes the chances of good perinatal outcome.

In summary, Kell sensitization found in 0.1% of pregnancies has a high chance of poor perinatal outcome (38%) when the baby is Kell positive. Appropriate evaluation of maternal anti-Kell titers is necessary with amniocentesis and  $\Delta OD_{450}$  determinations if the titer is  $\geq 1:32$ . Interpretation of  $\Delta OD_{450}$  results and subsequent management of the Kell-sensitized pregnancies cannot be based on the experience with rhesus sensitization.

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# Role of intravenous nitroglycerin in the treatment of severe pregnancy-induced hypertension complicated by pulmonary edema

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Intravenous nitroglycerin would appear to be an ideal agent for the treatment of severe pregnancy-induced hypertension complicated by cardiogenic pulmonary edema. Nitroglycerin infusion effectively reduces preload by venous dilatation and, at higher doses, results in arterial vasodilatation. Because of these pharmacologic properties, the effects of intravenous nitroglycerin were studied in three patients with severe pregnancy-induced hypertension complicated by pulmonary edema. The major cardiovascular effects of nitroglycerin were to reduce the mean pulmonary capillary wedge pressure from  $27 \pm 4$  to  $14 \pm 6$  mm Hg, which result in a change in the colloid osmotic pressure to pulmonary capillary wedge pressure gradient from  $-10$  to  $2$  mm Hg. No significant changes occurred in heart rate, central venous pressure, or cardiac index. Analysis of oxygen-related parameters revealed a significant ( $p < 0.05$ ) increase in oxygen delivery and extraction accompanied by a 53% increase in oxygen consumption. The changes in oxygen-related variables appeared to be secondary to a fall in mixed venous oxygen tension from  $39 \pm 4$  to  $33 \pm 1$  torr. These changes occurred without any significant improvement in arterial oxygen tension. We conclude that while intravenous nitroglycerin expeditiously corrects the hydrostatic derangements of pulmonary edema seen in pregnancy-induced hypertension, a rapid improvement in arterial oxygenation does not occur. (AM J OBSTET GYNECOL 1986;154:91-3.)

**Key words:** Intravenous nitroglycerin, pregnancy-induced hypertension, pulmonary edema

The management of pulmonary edema in severe pregnancy-induced hypertension has traditionally relied on oxygen supplementation, diuresis, and occasionally digitalization. While both cardiogenic and non-cardiogenic mechanisms have been implicated in this disease entity,<sup>1,2</sup> the treatment has remained essentially the same.

Intravenous nitroglycerin would appear to be an excellent agent for the treatment of pulmonary edema associated with pregnancy-induced hypertension, particularly that of a cardiogenic origin. Nitroglycerin acts to decrease preload and, at higher doses, to decrease afterload.<sup>3</sup> These properties should act to normalize the cardiovascular aberrations seen in this situation. When nitroglycerin is used for the control of severe hypertension, a rapid decline in pulmonary capillary wedge pressure precedes the expected drop in systemic arterial pressure. A possibility we considered is that an expeditious normalization of elevated pulmonary vas-

cular pressures would likewise result in a rapid improvement in oxygenation. We therefore investigated the efficacy of nitroglycerin in the treatment of severe pregnancy-induced hypertension complicated by pulmonary edema.

## Material and methods

Three antepartum patients with severe pregnancy-induced hypertension as defined by the American College of Obstetricians and Gynecologists were studied. Pulmonary edema was present both clinically and radiographically. After written informed consent was obtained, radial artery cannulation and right heart catheterization were accomplished. Approximately 15 minutes was allowed after placement of catheters to allow stabilization of cardiovascular parameters. A baseline set of hemodynamic measurements was then obtained, including arterial and mixed venous blood gases. A nitroglycerin infusion was started at  $6 \mu\text{g}/\text{min}$  and titrated to decrease the mean systemic arterial pressure by 20%. The goal of a 20% reduction in mean arterial pressure was arbitrarily chosen because in our experience a  $>20\%$  reduction often increases the risk of developing fetal heart rate abnormalities. Fifteen minutes after stabilization at the desired arterial pressure, a repeat set of hemodynamic and blood gas measure-

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**Table I.** Hemodynamic effects of intravenous nitroglycerin in pregnancy-induced hypertension complicated by pulmonary edema

	Before nitroglycerin infusion				After nitroglycerin infusion			
	Case 1	Case 2	Case 3	Mean $\pm$ SD	Case 1	Case 2	Case 3	Mean $\pm$ SD
Heart rate (bpm)	120	92	104	105 $\pm$ 11	122	105	93	107 $\pm$ 12
Mean arterial pressure (mm Hg)	174	140	130	148 $\pm$ 19	137	112	125	125 $\pm$ 10
Central venous pressure (mm Hg)	5	10	13	9 $\pm$ 3	5	3	13	7 $\pm$ 4
Pulmonary capillary wedge pressure (mm Hg)	30	22	30	27 $\pm$ 4	12	7	22	14 $\pm$ 6
Colloid osmotic pressure to pulmonary capillary wedge pressure gradient (mm Hg)	-11.6	-6.8	-11.6	-10.0 $\pm$ 2.3	3.9	7.3	-4.0	2.4 $\pm$ 4.7
Cardiac index (L/min/m <sup>2</sup> )	3.51	4.49	3.14	3.71 $\pm$ 0.57	2.53	5.12	3.09	3.58 $\pm$ 1.1
Systemic vascular resistance index (dyne $\cdot$ sec/cm <sup>5</sup> $\cdot$ m <sup>2</sup> )	3865	2312	2977	3051 $\pm$ 636	4186	1702	2889	2926 $\pm$ 1014
Left ventricular stroke work index (gm $\cdot$ m $\cdot$ m <sup>2</sup> )	61	83	43	62 $\pm$ 16	37	74	49	53 $\pm$ 15

**Table II.** Changes in oxygen-related parameters after intravenous nitroglycerin

	Before nitroglycerin infusion	After nitroglycerin infusion
Arterial oxygen tension (torr)	79 $\pm$ 6	72 $\pm$ 8
Mixed venous oxygen tension (torr)	39 $\pm$ 4	33 $\pm$ 1
Oxygen delivery index (ml/min $\cdot$ m <sup>2</sup> )	606 $\pm$ 144	617 $\pm$ 171
Oxygen consumption index (ml/min $\cdot$ m <sup>2</sup> )	146 $\pm$ 30	223 $\pm$ 50
Oxygen extraction rate (%)	0.25 $\pm$ 0.06	0.37 $\pm$ 0.04
Shunt fraction (%)	28 $\pm$ 7	21 $\pm$ 4

Values are mean  $\pm$  SD.

ments was obtained. Throughout the study period, left uterine displacement was maintained by use of a wedge under the right hip. A magnesium sulfate infusion was administered at 1.5 to 2.5 gm/hr throughout the study period. In one patient, 10 mg of furosemide was initiated at the time of invasive monitoring when an arterial oxygen tension of 58 mm Hg was noted. In the other two patients diuretic therapy was not initiated until pulmonary capillary wedge pressure reduction with intravenous nitroglycerin was accomplished.

Paired Student's *t* tests were used for comparison of the results with a level of  $p < 0.05$  being considered significant. One of the three patients required termination of the study for fetal distress. At that time the mean arterial pressure had only decreased from 130 to 125 mm Hg and the pulmonary capillary wedge pressure had declined from 30 to 22 mm Hg. This patient's parameters contributed to the large standard deviations seen here.

## Results

The cardiovascular changes induced by intravenous nitroglycerin are shown in Table I. Nitroglycerin in-

fusion resulted in an average mean systemic arterial pressure decrease from 148 to 125 mm Hg ( $p < 0.05$ ). With this decrease, no significant change in maternal heart rate was seen. While the central venous pressure only declined from 9 to 7 mm Hg, there was a substantial decrease in the average pulmonary capillary wedge pressure, from 27 to 14 mm Hg after infusion. Only a 4% drop in cardiac index was seen with these changes. As can be seen in Table I, two patients initially presented with relatively depressed left ventricular stroke work indices and high systemic vascular resistance indices. The third patient presented with nearly normal values (83 gm-meter  $\cdot$  m<sup>2</sup> and 2312 dyne  $\cdot$  sec/cm<sup>5</sup>  $\cdot$  m<sup>2</sup>, respectively). Colloid osmotic pressure remained essentially unchanged throughout the study period (17.3  $\pm$  1.5 mm Hg before treatment, 16.1  $\pm$  0.1 mm Hg after treatment). The decrease in pulmonary capillary wedge pressure, however, resulted in a significant improvement of the mean colloid osmotic pressure to pulmonary capillary wedge pressure gradient from -10 to +2 mm Hg ( $p < 0.05$ ). Despite improvement of the colloid osmotic pressure to pulmonary capillary wedge pressure gradient, the systemic arterial oxygen tension did not improve significantly during the study period (see Table II). Oxygen delivery, consumption, and extraction all increased after infusion of nitroglycerin. An unexpected finding was a decrease from 25% to 21% in the calculated intrapulmonary shunt fraction.

## Comment

Colley et al.<sup>4</sup> investigated the effect of nitroglycerin in a dog model with oleic acid-induced pulmonary edema. Their results revealed an increase in pulmonary shunt and a decrease in arterial oxygenation after nitroglycerin. These effects were attributed to inhibition of hypoxic pulmonary vasoconstriction and perfusion of poorly ventilated lung segments. This was, however,

a noncardiogenic model for pulmonary edema. In our study no significant improvement in systemic arterial oxygen tension was found, despite an improvement in the cardiovascular parameters.

The findings of an increased oxygen extraction and consumption in the face of a decrease in pulmonary shunt raise some questions. The variables involved in these calculations did not change significantly except for the mixed venous oxygen tension. This variable showed a significant drop after nitroglycerin from a mean of 39 to 33 mm Hg, representing a 16% reduction. This occurred in the face of only a 4% decline in cardiac index. These changes could indicate improved perfusion of previously underperfused vascular beds.

In 1966, Ferrer et al.<sup>5</sup> studied 18 nonpregnant patients with heart disease. Their results indicated that the splanchnic circulation reacted to nitroglycerin by an overall vasoconstrictive effect. Therefore, splanchnic pooling resulting in improved oxygen uptake was probably not a contributing factor. Other areas where major pooling could influence oxygen utilization include the systemic venous and the uteroplacental circulation systems. The contribution of each of these systems individually to the overall increase in oxygen extraction seen in this study could not be calculated. However, when Craft et al.<sup>6</sup> studied a phenylephrine-induced hypertensive pregnant ewe model, an increase in uterine arterial perfusion was noted after nitroglycerin infusion. Therefore, improved uterine perfusion could be a factor in this aspect of the study.

In light of the fall in mixed venous oxygen tension, one other possibility should be considered. Oxygen uptake by the blood during passage through the lungs may have actually increased after nitroglycerin as manifested by a sustained systemic arterial oxygen tension despite a markedly lower mixed venous oxygen tension presenting to the lung.

The pulmonary edema in these three patients appeared to be primarily hydrostatic as evidenced by the initially high pulmonary capillary wedge pressure. The fact that only two of the three patients presented with depressed myocardial function suggests that the causes of pulmonary edema in pregnancy-induced hypertension are many. Considering the rarity of these patients,

multi-institutional studies will most likely be needed to clarify the pathogenesis of this disease process. Previous investigations<sup>7,8</sup> have suggested that when the colloid osmotic pressure to pulmonary capillary wedge pressure gradient is <4 mm Hg, patients with pregnancy-induced hypertension may be more susceptible to the development of pulmonary edema. In our study, although this gradient was improved, a significant increase in systemic arterial oxygen tension did not occur. This gradient may represent a factor in the development of pulmonary edema, but it appears that expeditious correction of any alterations in this relationship will not result in a rapid improvement in oxygenation.

Finally, we conclude that intravenous nitroglycerin is an efficacious agent for rapidly correcting the hemodynamic derangements seen in pregnancy-induced hypertension complicated by hydrostatic pulmonary edema. Further investigation is required to determine if a concomitant reduction in blood volume via diuresis shortens the time interval required to resolve hypoxemia.

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# Plasma immunoreactive $\beta$ -endorphin in exercise-associated amenorrhea

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During 2 hours of bed rest, plasma samples were taken at 15-minute intervals from nine women with exercise-associated amenorrhea and in 11 control women in the follicular phase of a normal menstrual cycle. Concentrations of immunoreactive  $\beta$ -endorphin, cortisol, prolactin, luteinizing hormone, follicle-stimulating hormone, and estradiol were determined. During the first hour, cortisol levels decreased significantly in both groups and reached a plateau during the second hour, which period was considered to represent resting levels of the hormones. The amenorrhea group showed higher mean ( $\pm$  SE) resting levels of immunoreactive  $\beta$ -endorphin ( $11.0 \pm 0.8$  versus  $8.3 \pm 0.6$  pg/ml,  $p < 0.05$ ) and cortisol ( $274 \pm 35$  versus  $134 \pm 14$  nmol/L,  $p < 0.001$ ) and lower mean resting levels of prolactin ( $2.4 \pm 0.3$  versus  $5.7 \pm 1.1$  ng/ml,  $p < 0.01$ ), luteinizing hormone ( $4.0 \pm 0.7$  versus  $10.5 \pm 1.8$  IU/L,  $p < 0.01$ ), and estradiol ( $0.09 \pm 0.01$  versus  $0.19 \pm 0.07$  nmol/L,  $p < 0.05$ ) than the control group. These results suggest that exercise increases basal endorphin secretion in amenorrheic women and support the theory that increased opioid activity may be involved in the pathophysiology of exercise-associated amenorrhea. (Am J OBSTET GYNECOL 1986;154:94-7.)

**Key words:**  $\beta$ -Endorphin, exercise, amenorrhea

An increasing number of women are taking part in various sport activities in order to maintain good health. Strenuous exercise programs may, however, cause delayed menarche<sup>1</sup> and amenorrhea,<sup>2</sup> especially in combination with weight loss.<sup>3</sup> During a strenuous training period plasma levels of gonadotropins and estradiol decrease,<sup>4</sup> and these hormonal changes result in anovulation and menstrual disturbances. The exercise-associated amenorrhea is generally considered "hypothalamic" in origin, but the exact mechanism mediating the effect of exercise on the hypothalamic-pituitary function is poorly known.

$\beta$ -Endorphin and its precursor  $\beta$ -lipotropin are released from the anterior pituitary gland in response to stress, for instance, physical exercise.<sup>5-7</sup>  $\beta$ -Endorphin has been shown to inhibit gonadotropin secretion at the hypothalamic level.<sup>8</sup> Repeated increases in endogenous opioid activity in association with exercise have been suggested to be a mediating factor.<sup>6,7</sup> The aim of the present study was to reveal whether women with exercise-associated amenorrhea show any evidence of increased basal secretion of opioid peptides.

## Material and methods

**Subjects.** We studied nine women participating in various exercise programs who attended this clinic because of primary or secondary amenorrhea. Their ages varied from 15 to 18 years (mean  $\pm$  SD,  $16.7 \pm 1.1$  years). They practiced jogging, jazz ballet, or skating for 1 to 2 hours two to six times a week. One of them, who was 16 years of age, had primary amenorrhea. For the remaining subjects the menarcheal age varied from 11 to 15 years, after which they had had a period of amenorrhea of 9 to 30 months. Their weight varied from 85% to 95% of the ideal body weight determined in a large group of Finnish women. Preliminary studies revealed a low level of estradiol and low or normal values of luteinizing hormone and prolactin in plasma, suggesting a functional hypothalamic cause of amenorrhea. Eleven control subjects who did not take part in exercise programs and who had regular menstrual cycles were also recruited for the study. Their ages varied from 16 to 25 years ( $21 \pm 3.2$  years), and weights were 95% to 110% of their ideal body weight. None of the subjects in the study had been on a regimen of contraceptive steroids or any other hormonal therapy for at least half a year before the study.

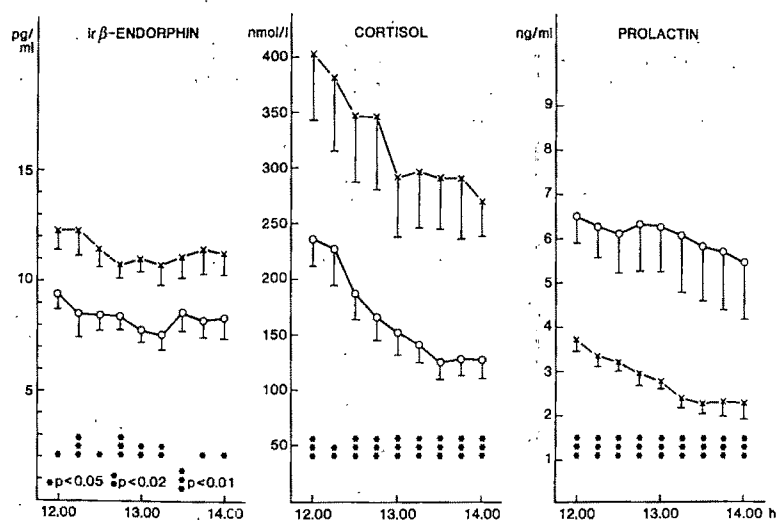
**Collection of blood samples.** The subjects had breakfast at 8.00 to 9.00 AM without tea or coffee and fasted afterward. An indwelling butterfly cannula was inserted into the vein of the forearm at noon, and 10 ml blood samples were drawn at 15-minute intervals for 2 hours into polyethylene tubes containing 1 mg of

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**Fig. 1.** Mean concentrations (SE) of immunoreactive  $\beta$ -endorphin, cortisol, and prolactin evaluated for 2 hours at bed rest in the group of nine women with exercise-associated amenorrhea (crosses) and in the group of 11 control women at the follicular phase of the normal cycle (open circles).

ethylenediaminetetraacetic acid per 1 ml of blood. The subjects were lying in bed. The tubes were immediately chilled on ice and centrifuged; 200  $\mu$ l of 1.6% (v/v) glycine in 1N hydrochloric acid was added per milliliter of plasma in order to prevent precipitation of proteins at thawing, and plasma was stored at  $-18^{\circ}\text{C}$  until analyzed. Serum samples were separately collected for the assays of prolactin, luteinizing hormone (LH), follicle-stimulating hormone (FSH), cortisol, and estradiol.

**Radioimmunoassay procedures.** Plasma endorphins were extracted as described by Cahill et al.<sup>9</sup> using Sep Pak  $\text{C}_{18}$  cartridges (Waters Associates Inc., Milford, Massachusetts), which were activated with 5 ml of methanol and 10 ml of distilled water before use. Samples of 2 ml of plasma, which had been acidified at the sample collection, were slowly applied to the cartridge by means of a suction apparatus (Vac-Elut, Analytichem International, Harbor City, California) and washed with 10 ml of distilled water and 10 ml of 4% acetic acid, and the peptides were eluted with 4 ml of a mixture of acetic acid and 90% ethanol (1:24). The samples were evaporated to about 0.5 ml with a rotatory vacuum concentrator and freeze-dried. The radioimmunoassay buffer contained, per liter, 0.2 mol of disodium acid phosphate/monosodium acid phosphate (pH 6), 1.5 gm of gelatin, 7.7 mmol of  $\text{NaN}_3$ , 10 gm of bovine serum albumin, and 0.8 ml of Triton X-100. The rabbit anti-serum "K<sub>2</sub>" given to us by Dr. J. Leppäluoto, Oulu, Finland, cross reacted on a molar basis 100% with  $\beta$ -endorphin and  $\beta$ -lipotropin but  $< 0.01\%$  with enkephalins and adrenocorticotrophic hormone.<sup>10</sup> The anti-serum was diluted 5000-fold in radioimmunoassay buffer. The lyophilized sample was dissolved in 100  $\mu$ l of radioimmunoassay buffer; 10,000 cpm of iodine

125-labeled  $\beta$ -endorphin in 100  $\mu$ l of radioimmunoassay buffer and 100  $\mu$ l of antiserum were added, and the mixture was incubated at  $4^{\circ}\text{C}$  for 48 hours. The antigen-antibody complexes were precipitated with the use of an anti-rabbit solid-phase antibody-coated cellulose suspension (Sac Cel, Wellcome Research Lab., Beckenham, England) according to the instructions given by the manufacturer. After centrifugation, the supernate was removed by aspiration and the radioactivity in the precipitate was counted. The intra-assay variation of the determination of immunoreactive  $\beta$ -endorphin was 11%. The recoveries of the reference  $\beta$ -endorphin and  $\beta$ -lipotropin added to plasma and carried through the procedure were 78% and 77%, respectively. Commercial kits for the assay of cortisol and prolactin were purchased from Farnos Diagnostics (Turku, Finland), those for the assays of LH and FSH from Amersham International Ltd. (Amersham, United Kingdom), and that for the assay of estradiol from Eir Ria (Würenlingam, Switzerland).

**Statistical analysis.** Analysis of variance for repeated measurements was performed with the use of Program P4V of BMDP Statistical Software Inc. (California). Significance of differences between the groups at each time point was separately tested by Student's *t* test.

## Results

Fig. 1 shows the mean plasma concentrations of immunoreactive  $\beta$ -endorphin, cortisol, and prolactin in the amenorrhea and control groups. The cortisol level fell significantly during the first hour ( $p < 0.0001$ ) and reached a plateau during the second hour. This fall was similar in both groups, but the mean level of cortisol was significantly higher in the amenorrhea group

**Table I.** Mean concentrations of immunoreactive  $\beta$ -endorphin, cortisol, prolactin, gonadotropins, and estradiol in plasma during the second hour of bed rest (resting levels) in exercise-associated amenorrhea and in control subjects at the follicular phase of the regular cycle

Hormone	Exercise-associated amenorrhea (N = 9)	Control subjects (N = 11)
Immunoreactive $\beta$ -endorphin (pg/ml)	11.0 $\pm$ 0.82*	8.3 $\pm$ 0.56*
Cortisol (nmol/L)	274 $\pm$ 35†	134 $\pm$ 14†
Prolactin (ng/ml)	2.4 $\pm$ 0.28‡	5.7 $\pm$ 1.1‡
LH (IU/L)	4.05 $\pm$ 0.69‡	10.5 $\pm$ 1.8‡
FSH (IU/L)	4.4 $\pm$ 0.41	4.9 $\pm$ 0.66
Estradiol (nmol/L)	0.09 $\pm$ 0.01*	0.19 $\pm$ 0.07*

Values are mean  $\pm$  SE.

\* $p < 0.05$ .

† $p < 0.001$ .

‡ $p < 0.01$ .

than in the control group ( $p = 0.005$ ). The slight decrease in the plasma level of immunoreactive  $\beta$ -endorphin was not statistically significant, the mean level being higher in the amenorrhea group than in the control group ( $p = 0.002$ ). The mean prolactin level was higher in the control group than in the amenorrhea group ( $p = 0.011$ ). Prolactin showed a significant decrease ( $p = 0.0006$ ) without any difference between the groups in relation to time.

Table I shows the mean resting levels of the hormones studied. The mean value for each subject was calculated with the use of the last four values of the study period. In the amenorrhea group the mean values of immunoreactive  $\beta$ -endorphin and cortisol were higher and the mean values of prolactin, LH, and estradiol were lower than those in the control group. In the total group of 20 subjects, a positive correlation was found between the resting levels of cortisol and immunoreactive  $\beta$ -endorphin ( $r = 0.68$ ;  $p < 0.01$ ), and a negative correlation between immunoreactive  $\beta$ -endorphin and LH ( $r = 0.66$ ;  $p < 0.05$ ). No significant correlation was found between the levels of immunoreactive  $\beta$ -endorphin and FSH and between immunoreactive  $\beta$ -endorphin and prolactin. The ratio of the resting level of cortisol (nanomoles per liter) to that of prolactin (nanograms per milliliter) completely separated the amenorrhea patients and the control subjects; this ratio varied from 7.4 to 52 in the control subjects and from 66 to 303 in the amenorrhea patients.

#### Comment

Both the control subjects and the patients were probably stressed before and at the beginning of the study but relaxed thereafter, which explains the significant

decrease in cortisol secretion during the first hour of the study period. The plasma immunoreactive  $\beta$ -endorphin level did not change significantly, but a possibility remains that it was increased before the start of the study. The present results suggest that both endorphin and cortisol secretion are increased in patients with exercise-associated amenorrhea when compared with control subjects in the follicular phase of the cycle. Our results are in agreement with those of Russel et al.,<sup>4</sup> who found an increased resting serum level of  $\beta$ -endorphin immunoreactivity in women who were training in strenuous swimming and had menstrual disturbances. Previous findings of the effects of training on the release of  $\beta$ -endorphin in response to a course of exercise are conflicting; Carr et al.<sup>6</sup> and Bullen et al.<sup>11</sup> found an increase whereas no significant change could be found by Howlett et al.<sup>7</sup> There is experimental evidence that in rats chronic stress increases the content of  $\beta$ -endorphin and corticotropin in the anterior pituitary in a resting state and that chronic stress increases the release of  $\beta$ -endorphin in response to acute stress.<sup>12</sup>

In untrained subjects, the ergometer test was not found to elicit any significant increase in the plasma prolactin level, but in the course of the training prolactin secretion increased in response to the ergometer test.<sup>11,13</sup> This is not reflected in the basal secretion of prolactin as indicated by decreased resting levels of prolactin in the present amenorrhea patients and in female athletes studied by Russel et al.<sup>4</sup> Thus, although hyperprolactinemia is known to be a factor mediating menstrual disturbances,<sup>14</sup> it does not seem to be a significant factor in exercise-associated amenorrhea.

In the hypothalamus,  $\beta$ -endorphin inhibits the pulsatile release of gonadotropin-releasing hormone.<sup>15</sup> In women with hypothalamic amenorrhea<sup>16</sup> and in amenorrheic runners<sup>17</sup> naloxone infusion was found to increase the pulsatile secretion of LH, suggesting that hypothalamic opioid peptides inhibited LH secretion in these subjects. This shows that the increase in opioid activity associated with training is not limited to their increased secretion by the pituitary but extends to the hypothalamic level. In the present study, we found a negative correlation between plasma levels of immunoreactive  $\beta$ -endorphin and LH. Thus evidence is accumulating that endogenous opioid peptides may mediate between "stress" and disturbances of the menstrual cycle in exercising women. The nature of this stress is, however, obscure. A psychic component was suggested by Schwartz et al.,<sup>18</sup> who found that amenorrheic runners associated more psychic stress with their running than did the runners that were menstruating regularly.

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# Adjunctive erythromycin treatment for idiopathic preterm labor: Results of a randomized, double-blinded, placebo-controlled trial

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Pathogenesis and optimal treatment and prevention of preterm labor remain incompletely understood. Entry of cervical/vaginal microorganisms into lower uterine tissues has been implicated in preterm labor and may be amenable to specific therapy. Fifty-eight women with <34 completed weeks of gestation and without other obstetric complications, who were receiving intravenous tocolytics because of uterine contractions and who had cervical alteration (<5 cm dilated), were enrolled in a prospective randomized, double-blinded evaluation of 7 days of adjunctive therapy with enteric-coated erythromycin base (333 mg three times daily by mouth) versus placebo. Microbiologic examination included cultures for *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, and group B streptococcus. Fifty-eight women with singleton pregnancies (29 erythromycin; 29 placebo) completed the protocol. Among women with cervical dilatation  $\geq 1$  cm at the beginning of treatment, mean time until delivery was 32.5 days with erythromycin and 22.4 days with placebo treatment ( $p = 0.027$ ). Of the erythromycin-treated women, seven of eight were delivered at  $\geq 37$  weeks and only three of nine placebo-treated women were delivered at  $\geq 37$  weeks ( $p = 0.035$ ). Orally administered enteric-coated erythromycin as adjunctive treatment of pregnant women in labor  $\leq 34$  weeks is well tolerated. Adjunctive erythromycin given to women treated for preterm labor  $\leq 34$  weeks is associated with prolongation of pregnancy and delivery at 37 weeks only in women with cervical dilatation at the beginning of treatment. (AM J OBSTET GYNECOL 1986;154:98-103.)

**Key words:** Preterm labor, treatment, antibiotics, erythromycin

Birth weight is the single most important determinant of a newborn infant's chance for survival and healthy growth and development.<sup>1,2</sup> Within industrialized countries, short gestation resulting from preterm labor and delivery accounts for most of the 6% to 7% of newborn infants with low weight (<2500 gm) at birth.<sup>1</sup> Infants weighing <1500 gm account for 65% of neonatal mortality.<sup>3</sup> Preterm birth results in immense individual, familial, and societal costs. Expenses for direct care alone for such infants are more than 1 billion dollars per year in the United States alone.<sup>1</sup>

There are multiple causes of preterm labor. Individual pathophysiologic mechanisms must be defined before cause-specific intervention can be implemented. Maternal medical and anatomic factors, such as cervical incompetence, multiple gestation, and uterine abnormalities, are causally linked to short gestation.<sup>3</sup> In the

majority of patients, however, pathophysiologic mechanisms remain poorly understood. Much data point to infection as an unappreciated cause of preterm labor and birth. Numerous authors, most prominently Knox, Driscoll, Benirschke, and Naeye, have detailed observations demonstrating a strong association between clinical or subclinical infection and inflammation within the uterus and the process of preterm birth.<sup>4</sup> Recently, these microbiologic and histologic findings have been summarized.<sup>5,6</sup> Despite persuasive circumstantial evidence, the association between infection and preterm birth is doubted by others because the vaginal flora is usually normal and plausible pathophysiologic mechanisms have not been recognized.<sup>7</sup>

It is now possible to correlate intrauterine findings of inflammation with microbiologic recovery of various cervical/vaginal microflora and pathophysiologic mechanisms that can cause preterm labor. We have demonstrated that multiple constituents of the female genital tract microflora produce putative virulence factors including protease(s), collagenases, and elastases, along with phospholipase C (McGregor JA, Todd JK, Lawellin D, et al. Protease production in reproductive tract microorganisms, unpublished data. McGregor JA, Lawellin D, Franco-Buff A, et al. Phospholipase C

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**Table I.** Demographic, historical, obstetric, and perinatal parameters evaluated

<b>Maternal</b>	
1. Age	16. Fetal anomalies
2. Race	17. Frequency of coitus
3. Insurance rating	18. Sexually transmitted disease
4. Gravidity	19. Readmission for preterm labor
5. Parity	20. Cervical microbiologic findings
6. Weeks of antenatal care	21. Premature rupture of membranes
7. Gestational age at start of study	22. Induced labor
8. Cervical dilatation	23. Cesarean section
9. Cervical effacement	24. Treated urinary tract infection with positive culture
10. Multiple gestation	25. Treated urinary tract infection with negative culture
11. Prior preterm birth	26. Leukocyte count at onset of study
12. Amniocentesis, before treatment	27. Leukocyte count at delivery
13. Amniocentesis, after treatment	28. Days in hospital
14. Fetal position	29. Days on regimen of oral terbutaline
15. Steroid treatment	30. Days to delivery
<b>Perinatal</b>	
1. Perinatal mortality	6. Neonatal sepsis suspected (negative blood culture)
2. Birth weight	7. Course of antibiotics
3. Gestational age (Dubowitz)	8. Days in level I nursery
4. Apgar score (5 min)	9. Days in level II or III nursery
5. Amonalies	10. Neonatal sepsis confirmed

production by reproductive tract microorganisms, unpublished data). We have reconfirmed the demonstration by Bejar et al.<sup>8</sup> of phospholipase A<sub>2</sub> activity among similar organisms (McGregor JA, Lawellin D, Franco-Buff A, et al. Phospholipase A<sub>2</sub> activity among reproductive tract microorganisms evaluated with two substrates, unpublished data). We have also demonstrated that subinhibitory levels of various antimicrobials reduce protease production by selected cervical/vaginal organisms (McGregor JA, Franco-Buff A, Todd JK, et al. Protease production inhibition by sub-MIC antimicrobial levels in reproductive tract microorganisms, unpublished data). These and additional findings suggest that pathophysiologic processes initiated by cervical/vaginal microorganisms can be associated with preterm labor in some women and may be reversed by appropriate antibiotic therapy.

Consequently, we conducted a placebo-controlled clinical trial to evaluate erythromycin as adjunctive therapy for women with preterm labor who did not present with recognized causal factors of preterm labor. Enteric-coated erythromycin base (333 mg of E-mycin, The Upjohn Company, Kalamazoo, Michigan) was chosen as an antimicrobial probe for the following reasons: (1) Erythromycin base is bacteriostatic for the majority of aerobic and anaerobic cervical/vaginal microflora and appears safe in pregnancy<sup>9, 10</sup>; (2) antimicrobials similar to erythromycin are effective in reducing production or release of protease and other extracellular products by a variety of microorganisms<sup>11</sup>; (3) erythromycin crosses into the placental-fetal unit poorly and is unlikely to interfere with recognition and evaluation of amniotic fluid infection<sup>12</sup>; (4) even in the presence of high tissue concentrations, erythromycin is poorly excreted in vaginal fluid and is unlikely to lead to over-

growth of resistant organisms within the lower genital tract<sup>13</sup>; (5) 333 mg of erythromycin base given three times a day is a convenient and well-tolerated formulation that achieves adequate blood and tissue concentrations in humans<sup>12, 13</sup>; and (6) there is no suggestion in available literature that erythromycin has any independent tocolytic or antiprostaglandin effect.

#### Material and methods

A prospective, randomized, double-blinded, placebo-controlled study of enteric-coated erythromycin base (333 mg of E-Mycin) given three times a day orally for a 7-day course versus placebo was carried out in pregnant women at University Hospital, University of Colorado Health Sciences Center, Denver, Colorado. Criteria for inclusion were (1) clinical diagnosis of preterm labor with initiation of parenteral tocolytic treatment (either terbutaline or magnesium sulfate), (2) completion of no more than 34 weeks' gestation as determined by clinical criteria and ultrasound fetal measurements, (3) cervical dilatation of no more than 4 cm, (4) age of 18 years or older or an emancipated minor, and (5) completion of an informed consent form.

Immediate exclusions included (1) diagnosis of known cause of preterm labor (that is, preterm rupture of membranes, uterine abnormalities, multiple gestation, placental accident, etc.), (2) presence of any condition requiring antimicrobial therapy (that is, urinary tract infection, chorioamnionitis, clinical vaginitis, or cervicitis, etc.), (3) prior history of allergy or intolerance to erythromycin base, (4) inability to take oral medication, and (5) unwillingness to complete a 7-day regimen.

Late exclusions were determined before study drug codes were broken and included (1) inability to com-



**Table II.** Late exclusion from study

	Erythromycin (N = 29)	Placebo (N = 29)
Incomplete course		
Unspecified reason	1	0
Delivery	2	2
Nausea	1	1
Lost to follow-up	1	1
Treatment for urinary tract infection (culture positive)	1	0
Treatment for urinary tract infection (culture negative)	1	2
Twins	4	4
Patients remaining after exclu- sions	18	19
Remaining patients with cervix dilated $\geq 1$ cm	8	9

plete 7 days of drug treatment; (2) intolerance of erythromycin base or placebo; (3) subsequent treatment with an antibiotic, most commonly for suspected urinary tract infection; (4) twin gestations; and (5) patients who were lost to follow-up.

We evaluated more than 40 measures of patient history (demographic, reproductive, perinatal, and sexual) and pregnancy outcome. Follow-up data were obtained by personal visits, record reviews of delivery and newborn admissions from both local and hometown hospitals where delivery occurred, and telephone calls to private physicians and the study patients themselves. Gestational age was determined by Dubowitz scoring.

Enteric-coated erythromycin base tablets were prepared without markings by The Upjohn Company. Drug-containing tablets were identical with lactose-filled placebo tablets and came in identical coded bottles. Bottles contained 21 tablets, sufficient for 7 days of treatment. Patient assignment to treatment with erythromycin or placebo was determined by a computer-generated randomized list of numbers. Codes were kept sealed by the manufacturer and by the chief pharmacist. Patients were seen daily and phoned at home after discharge to ensure completion of treatment.

Upon admission to the study, the cervix was examined and cultured for *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, group A and group B streptococci, herpes simplex virus, and cytomegalovirus. Specimens were inoculated on appropriate culture media at the bedside and microorganisms were identified according to standard microbiologic techniques in the Clinical Microbiology Laboratory, University of Colorado Hospital. Amniocentesis with microscopic and microbiologic examination of amniotic fluid was not a criterion for admission to the protocol but was frequently performed. Fisher's exact test was used for analysis of dichotomous data and Student's *t* test was used for continuous data.

One-tailed tests were used to test the hypothesis that treatment with erythromycin would be associated with lengthened gestation and increased birth weight.

## Results

Fifty-eight patients completed the protocol. Overall, the 29 women given erythromycin and the 29 women administered placebo did not differ clinically or statistically in any demographic, reproductive, sexual behavior, obstetric, and/or perinatal category evaluated. These 40 parameters for comparison are listed in Table I. There were no differences in tobacco use in any comparison group.

Mean duration of continued pregnancy was 40 days in the erythromycin-treated women and 34 days in the placebo-treated women. Birth weight averaged 115 gm more in the antibiotic group than in the placebo-treated group. Three erythromycin-treated women with singleton gestations had rupture of the membranes before the onset of labor at 19, 22, and 23 days after starting therapy. Two similar placebo-treated women suffered premature rupture 5 and 9 days after starting treatment. None of these differences was statistically significant. Lack of statistical difference in any measured parameter when all single pregnancies were examined may be explained primarily by either lack of drug effect, insufficient drug dose or length of administration, insufficient numbers of women examined to establish small differences, and/or insufficient clinical diagnostic criteria for preterm labor.

Hendricks<sup>14</sup> pointed out that about half of patients clinically identified as being in preterm labor subsequently carry the pregnancy "substantially further." He suggested that women with cervical changes are more likely to be in preterm labor and can benefit from effective treatment. It is also the case that cervical/vaginal microorganisms are most likely to ascend and exert an untoward effect through an effaced and dilated cervix. With these points in mind, we compared only study patients with evidence of cervical effacement and dilatation  $\geq 1$  cm for drug effect. After the exclusions noted in Table II (incomplete course, antibiotic use, lost to follow-up, twins), there remained eight and nine women with cervical changes in the antibiotic- and placebo-treated groups, respectively. Analysis of selected parameters for these study women and perinates is contained in Tables III to V. There were no demographic or historic statistical differences except for a mean of 1.9 and 2.9 total pregnancies in the erythromycin- and placebo-treated groups, respectively. Mean cervical dilatation was 1.6 and 1.5 cm at the beginning of treatment in the erythromycin- and placebo-treated groups, respectively. Among these women, there was statistically significant prolongation of pregnancy with a greater number of days from start of treatment until

**Table III.** Selected demographic and obstetric parameters for women with cervical effacement and dilatation  $\geq 1$  and  $< 5$  cm

Parameter	Erythromycin (N = 8)		Placebo (N = 9)		p Value
	Mean	Range	Mean	Range	
Age (yr)	21.6	17-29	22.2	16-33	NS
Gravidity	1.88	1-4	2.88	1-8	0.15
Previous preterm birth	4/8		2/9		0.22
Gestational age at start of study (wk)	32.9	31-34	33.0	30-34	NS
All other categories	No clinical or statistical differences				

**Table IV.** Selected obstetric outcome parameters of study women with cervical effacement and dilatation  $\geq 1$  and  $< 5$  cm

	Erythromycin	Placebo	p Value
Days, start of treatment to delivery	32.5 $\pm$ 11.24 (19-39)*	22.4 $\pm$ 7.24 (9-29)	0.027
Delivery at 37 wk	7/8	3/9	0.035
Mothers readmitted for preterm labor (excluding delivery)	3/8	6/9	0.24
Amniocentesis	6/8	3/5†	NS
Maternal days in hospital (initial admission)	6.1 $\pm$ 4.7 (3-15)*	6.3 $\pm$ 6.2 (2-18)*	NS
Amniotic fluid infection	0/8	0/9	NS
Maternal febrile morbidity	0/8	0/8	NS

\*Mean  $\pm$  standard deviation and range.

†Unsuccessful in one patient.

**Table V.** Selected perinatal outcomes in women with cervical effacement and dilatation  $\geq 1$  and  $< 5$  cm

	Erythromycin	Placebo	p Value
Birth weight	2943 $\pm$ 483.8 (2340-3572)*	2615 $\pm$ 459.1 (1550-3060)	0.07
Initial requirement of neonate			
Intensive care nursery	0	2	NS
Intermediate care nursery	2	1	NS
Low-risk nursery	6	6	NS
Total days in intensive care or intermediate nursery	9	62†	—
Total days in nursery	3 $\pm$ 2.1*	9.6 $\pm$ 13.5*	0.08
Treated with antibiotics	0/8	1/9‡	—

\*Mean  $\pm$  standard deviation and range.

†Two preterm newborn infants.

‡Negative culture.

delivery (mean = 32.5 in the erythromycin group versus 22.4 in the placebo group;  $p = 0.027$ ) and delivery at or beyond 37 weeks (Tables III and IV). Of patients who completed erythromycin treatment, seven of eight were delivered at  $\geq 37$  weeks' gestation and only three of nine placebo-treated women were delivered at  $\geq 37$  weeks' gestation ( $p = 0.03$ ). Strong statistical trends toward greater birth weight ( $p = 0.07$ ), reduced total nursery days ( $p = 0.08$ ), and reduced maternal readmissions for preterm labor ( $p = 0.24$ ) were noted in the drug-treated group (Tables IV and V). Newborn infants of erythromycin-treated women weighed an average of 328 gm more than those of placebo-treated women and required intensive or intermediate nursery care for 9 days versus 62 days with placebo treatment (Table V).

Discovery of acknowledged reproductive tract patho-

genic microorganisms was infrequent. Single recoveries of *C. trachomatis* and *Streptococcus agalactiae* were made from one woman within the placebo group and one woman within the erythromycin group. These two patients were delivered before the microbiologic diagnosis was made. Neither of these patients was specifically treated before delivery. There was no recovery of herpes simplex virus, cytomegalovirus, or *N. gonorrhoeae* in any study patient. Among a total of 12 amniocenteses, there was only a single isolation with recovery of *Gardnerella vaginalis* from an erythromycin-treated patient with twins. This patient received antibiotic therapy.

Enteric-coated erythromycin base (333 mg of E-mycin) was well tolerated. One drug-treated woman and one placebo-treated woman withdrew from the study because of nausea and/or vomiting.

### Comment

We have used oral erythromycin base treatment to probe pathophysiologic factors associated with unexplained preterm labor. Statistically significant benefits limited to women with cervical effacement and dilatation were found. These findings support our hypothesis that elements of cervical/vaginal microflora may gain access to the lower uterine segment and directly or indirectly provoke the process of preterm labor. Others have suggested that the finding of *S. agalactiae*, *C. trachomatis*, *T. vaginalis*, or the observation of bacterial vaginosis or *Gardnerella*-associated nonspecific vaginitis places patients at increased risk for preterm labor.<sup>15-17</sup> Preterm labor also occurs in the course of generalized uteroplacental and fetal infection with a variety of organisms including *Listeria monocytogenes*.<sup>4</sup>

The presence of a specific "pathogen" within the reproductive tract may not necessarily be required to initiate the process of preterm labor. As noted, multiple biologic constituents of cervical/vaginal "normal flora" produce substances that may play either isolated, additive, or synergistic roles in bringing about the process of labor. Bejar et al.<sup>8</sup> described phospholipase A<sub>2</sub> production among numerous microbial species that occur in the lower genital tract. These findings have been confirmed through the use of radiolabeled arachidonic acid substrates (McGregor JA, Lawellin D, Franco-Buff A, et al. Phospholipase A<sub>2</sub> activity among reproductive tract microorganisms evaluated with two substrates, unpublished data). Similarly, phospholipase C activity occurs in many of these same microorganisms, including many anaerobic species (McGregor JA, Lawellin D, Franco-Buff A, et al. Phospholipase C production by reproductive tract microorganisms, unpublished data). Phospholipase C in combination with endogenous diglyceride lipase releases arachidonic acid from phospholipid sources.<sup>18</sup> The presence of arachidonic acid is a rate-limiting factor in the production of prostaglandins such as E<sub>2</sub> and F<sub>2α</sub>.<sup>19</sup> Both these prostanoids are physiologically important in labor.<sup>20</sup> Recent observations show that microorganisms themselves may metabolize arachidonic acid into various eicosanoids (Lawellin D, McGregor JA, Kishiyama J, et al. Formation of arachidonic acid metabolites by microorganisms, unpublished data). Further, many cervical/vaginal microorganisms, as well as neutrophils and macrophages, produce a variety of proteases including collagenase and elastase, which can locally diminish the tactile strength of chorionic membranes in vitro<sup>21</sup> (McGregor JA, Todd JK, Lawellin D, et al. Protease production in reproductive tract microorganisms, unpublished data). Much previous work has demonstrated that lower genital tract microflora is found within amniotic fluid obtained by amniocentesis near term and in the lower uterine segment at cesarean section.<sup>22-24</sup>

Cervical dilatation, as well as other factors, may be important in allowing cervical/vaginal microorganisms to gain access through physical and biochemical host defenses at the cervix.<sup>25</sup> If this is the case, then it is likely that erythromycin treatment would have its greatest effect on women in whom the cervix is most open and a greater number of organisms is present in the lower uterine segment. A sufficient number of microorganisms within the lower uterine segment may produce "virulence factors" that may overcome physiologic control mechanisms and provoke onset of untimely labor. Alternatively, the presence of microorganisms and their extracellular products may incite a local inflammatory reaction within the lower uterine segment. Polymorphonuclear cells and macrophages, which are part of this reaction, release a variety of proteases and lipases, which may further the local release of arachidonic acid and cause disruption of easily traumatized, enzyme-laden decidual cells.<sup>26, 27</sup> Erythromycin treatment may interfere with these potentially self-reinforcing processes by hastening clearance of susceptible microorganisms within the lower uterine segment and/or by reducing production and release of damaging substances from susceptible bacteria.

Findings of lengthened gestation, fewer preterm births, and higher mean birth weights have been described in less well-controlled studies of erythromycin and tetracycline in pregnant women not in labor.<sup>28, 29</sup> Elder et al.<sup>28</sup> randomly treated 279 nonbacteriuric pregnant women for 6 weeks with 1 gm of oral tetracycline daily or placebo beginning before 32 weeks' gestation. The antibiotic-treated women had significantly fewer preterm deliveries and more completed weeks of gestation. Kass et al.<sup>29</sup> demonstrated increased birth weights in newborn infants among mothers treated with erythromycin versus placebo in the third trimester. These studies suggest that antimicrobial agents may act to reduce the risk of preterm labor and birth by preventing or modifying activities of susceptible microorganisms.<sup>28, 29</sup> This study tends to confirm the findings of Elder et al.<sup>28</sup> and Kass et al.<sup>29</sup> and demonstrates that antimicrobial treatment has an effect even when given in apparent preterm labor. The use of well-tolerated enteric-coated erythromycin base overcomes objections regarding poor tolerance of non-enteric-coated erythromycin and the contraindication of tetracycline use in pregnant women.

The potential individual and societal benefits of effective means of prolonging gestational length and avoiding the developmental, medical, and iatrogenic sequelae of preterm birth are potentially immense, even if applied only to a relatively small group of women and perinates. However, prolongation of gestation should not be viewed as an unqualified benefit if the perinate or mother is placed at increased risk



from an undetected adverse intrauterine or iatrogenic influence during continued pregnancy. If the fetus suffers from sepsis due to undetected chorioamnionitis or sustained placental insufficiency, then continued gestation may not be a perinatal benefit. Maternal risk from tocolytic therapy with  $\beta$ -sympathomimetic agents includes pulmonary edema and metabolic disturbances. Antimicrobial treatment is associated with maternal intolerance, hypersensitivity, organ-specific toxicity, superinfection, and changes in individual and community microecology. Any therapy for prolonging gestation in women threatened by idiopathic preterm labor should be evaluated fully so that presumed benefits are not outweighed by risks for mother and fetus. In this study, no untoward reaction occurred other than maternal nausea and vomiting, which occurred only once each in both erythromycin- and placebo-treated groups.

In conclusion, in this double-blinded, randomized, placebo-controlled trial, a 1 week course of orally administered erythromycin was associated with statistically significant benefit only in women with cervical dilatation  $\geq 1$  cm at the start of treatment. Erythromycin-treated women, as compared with placebo-treated women, had significantly greater prolongation of pregnancy with an increased number of days from the start of treatment until delivery at or beyond 37 weeks. There were also strong statistical trends toward greater birth weight, reduced nursery days, and reduced maternal readmissions for preterm labor in drug-treated women. Infants born after erythromycin treatment spent a total of 9 days in intensive or intermediate nurseries versus 62 days for those whose mothers took placebo. These data tend to confirm the observations of Elder et al.<sup>28</sup> and Kass et al.,<sup>29</sup> who suggested that the reduced risk of preterm labor was an effect of maternal antibiotic treatment. In order to confirm these findings and further delineate the possible mechanisms involved, additional microbiologic and clinical experiments are required.

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# Squamous cells in the maternal pulmonary circulation

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Identification of squamous cells in the maternal pulmonary arterial circulation, either at autopsy or in blood aspirated from a pulmonary artery catheter, is currently regarded as pathognomonic for amniotic fluid embolism. Sixteen pregnant women underwent pulmonary arterial catheterization for a variety of medical indications. Examination of the buffy coat fraction of the distal lumen aspirate resulted in the identification of squamous cells in all cases. Squamous cells were similarly identified in control specimens from 17 nonpregnant patients; however, the difference in cell count between the pregnant and nonpregnant patients was significant. Such cells presumably reflect, in part, bloodstream contamination from sites of venous access. Reliable differentiation of adult from fetal squamous cells is not possible; however, the significant increase in cell count documented in pregnant patients suggests a possible fetal origin for some squamous cells detected during pregnancy. The detection of squamous cells in the pulmonary arterial circulation of pregnant women is not pathognomonic for amniotic fluid embolism. In a critically ill obstetric patient, such a finding should not deter the clinician from a thorough search for other causes of hemodynamic instability. (AM J OBSTET GYNECOL 1986;154:104-6.)

**Key words:** Amniotic fluid embolism, fetal squamous cells, pulmonary artery catheter

Amniotic fluid embolism is an uncommon obstetric condition characterized by hypotension, hypoxia, and, in 40% of cases, disseminated intravascular coagulation.<sup>1</sup> Maternal mortality approaches 80%.<sup>2</sup> Detection of squamous cells in the pulmonary arterial circulation, either at autopsy or in blood aspirated from the distal lumen of a pulmonary arterial catheter, is traditionally regarded as being pathognomonic for this condition<sup>3-5</sup>; because of the diversity of the clinical presentation, such documentation is considered essential for diagnosis. This study was undertaken in order to define the frequency with which squamous cells may be isolated from the pulmonary arterial circulation of pregnant women.

## Methods

Between November, 1983, and July, 1984, 16 pregnant women underwent pulmonary arterial catheterization for a variety of medical indications. Proper placement was confirmed by waveform and chest x-ray film. In all cases, the route of insertion was the right internal jugular vein. Gestational ages ranged from 28 to 43 weeks. Samples were collected and prepared in an identical manner from 17 nonpregnant patients in medical and surgical intensive care units. Three milli-

liters of blood was aspirated from the distal catheter lumen and discarded. An additional 7 ml was then withdrawn and injected into a heparinized tube. The specimen was centrifuged and smears were made from the buffy coat layer. Standard Wright and Sudan Black preparations were then examined by one of us (Z. P.) from the Department of Pathology for detection of squamous cells. The solution for the Wright stain was delivered in a closed system, and automated slide preparation was used. Throughout the study, utmost care was taken to avoid any epidermal contact with the slide surfaces. In the final 18 patients, all slide handling was undertaken by a technician wearing both gloves and a hair net.

From each specimen, two Wright-stained smears from the buffy coat were examined for semiquantification of squamous cells. A count was made of all squamous cells identified for a distance of two low-power fields from the peripheral feathered-edge and one low-power field from the lateral margins. Only undistorted cells exhibiting the classic morphologic and staining characteristics of squamous cells were counted. Reported cell counts represent the mean for both slides. Following our initial observations, several slides were examined by an independent pathologist who confirmed identification of squamous cells.

## Results

Squamous cells were identified in every patient (Fig. 1). Table I details the medical complications of pregnant patients undergoing pulmonary arterial catheterization. Also listed is the mean number of squamous cells identified during the prepartum and postpartum

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Fig. 1. Wright stain preparation of pulmonary arterial blood of patient with term pregnancy and New York Heart Association Class IV mitral stenosis.

Table I. Pulmonary arterial catheterization in pregnant patients

Indication	Patients	Mean squamous cell count
Severe pregnancy-induced hypertension	6	8
Septic shock	3	14
Severe cardiac disease during labor	3	9
Unresponsive hypovolemic shock	2	10
Pulmonary edema, unknown cause	1	5
Amniotic fluid embolism	1	6

periods. Table II compares squamous cell quantification for pregnant patients during the prepartum and postpartum periods as well as for nonpregnant control subjects. In pregnant patients (excluding one patient with clinical amniotic fluid embolism), the mean ( $\pm$ SEM) number of squamous cells identified was  $7 \pm 1$  for antepartum specimens and  $12 \pm 3$  for samples collected during the postpartum period. This difference did not reach statistical significance. The semiquantitative cell count for the patient with eventually fatal amniotic fluid embolism was 6. The difference in mean cell count between the postpartum and nonpregnant patients was significant ( $p < 0.05$ ) with the use of a two-sample  $t$  test with the assumption of unequal variance.

#### Comment

In a study in healthy pregnant patients, Sparr and Pritchard<sup>6</sup> injected chromium-labeled erythrocytes into the amniotic cavity. Examination of peripheral blood failed to demonstrate the presence of labeled red blood cells. This experiment has been cited as evidence that embolization of amniotic fluid debris does not occur in normal pregnancy. Based on these data and the detection of squamous cells and other debris of presumed

Table II. Semiquantitative assessment of squamous cells collected by pulmonary arterial catheterization (mean  $\pm$  SEM)

Population	Cell count
Pregnant* (N = 15)	
Antepartum	$7 \pm 1$
Postpartum	$12 \pm 3$
Nonpregnant (N = 17)	$5 \pm 1^\dagger$

\*Excluding patient with clinical amniotic fluid embolism.

$^\dagger p < 0.05$ .

amniotic origin in the pulmonary vasculature of peripartum patients with the syndrome of hypotension, hypoxia, and coagulopathy, it has been presumed that such a finding is both necessary and sufficient for the diagnosis of amniotic fluid embolism.<sup>3-5</sup> Recently, in response to a case report of amniotic fluid embolism, a letter appeared describing two pregnant patients with other medical conditions in whom squamous cells were detected in pulmonary arterial blood.<sup>7</sup> We have demonstrated that squamous cells are almost universally detected in the pulmonary arterial circulation of pregnant patients.

There are several possible explanations for the seeming discrepancy between our findings and those of Sparr and Pritchard.<sup>6</sup> The latter study involved a series of artificially induced physiologic manipulations and measurements in an effort indirectly to examine the possible release of squamous cells into the maternal circulation. The possibilities of error in such a complex experimental design were discussed at length by the authors of this work. In their study, peripheral blood was examined at frequent intervals for up to 4 days following injection of chromium-labeled erythrocytes into the amniotic cavity. Assuming that asymptomatic embolization of amniotic fluid debris is a gradual pro-



cess, it seems possible that 4 days would be insufficient time to permit detectable embolization with the methods of this study. Certainly, the simple design of the present study, involving direct examination of pulmonary arterial blood, is much less susceptible to experimental error.

Every attempt was made to eliminate the possibility of artifact or contamination from this study. Strict adherence to the previously described criteria for squamous cell identification, as well as independent examination of the preparations by a pathologist from an unrelated institution, eliminates, in our opinion, the possibility of the former. The closed system stain delivery and mechanical slide preparation, in addition to meticulous handling of specimens by all individuals involved in the project, minimizes the latter possibility. A further procedural change late in the study to include gloves and a surgical hairnet during all slide handling did not alter the frequency with which squames were detected.

The release of adult epidermal cells into the circulation from the site of introducer sheath placement in all likelihood accounts for the significant number of squamous cells detected in nonpregnant patients. Squamous cells are commonly found in peripheral blood smears obtained by venipuncture and represent epidermal contamination. The fact that nonpregnant control specimens yielded a significant difference in cell count from that observed in postpartum patients, as well as the trend in pregnant subjects toward increasing numbers of squamous cells in postpartum samples, suggests that fetal cells may have contributed to the total cell count observed in pregnant patients. Unfortunately, reliable histologic differentiation of adult from fetal squamous cells is not possible; thus the fetal origin of such cells can only be inferred. However, regardless of the origin of these cells, the implication of this report remains the same: The detection of squamous cells in meticulously handled pulmonary arterial blood of critically ill pregnant patients is not sufficient for the diagnosis of amniotic fluid embolism.

It is well established that trophoblastic cells are commonly found in the maternal venous circulation.<sup>8</sup> One may postulate that small-scale embolization of fetal squamous cells is, in a similar manner, a common and generally benign phenomenon and that the symptoms commonly associated with amniotic fluid embolism occur only with larger volumes of debris. It was, therefore, surprising that we could not detect a quantitative difference in cell count between one patient with fatal amniotic fluid embolism and other pregnant patients. This finding corroborates data indicating a poor correlation between clinical symptoms and the volume of experimentally injected fetal debris and suggests that in clinical amniotic fluid embolism, such cells may possibly simply be a marker of a condition caused by an

abnormal substance within amniotic fluid.<sup>9-12</sup> Alternatively, this finding may reflect rapid clearance of squamous cells from the pulmonary arterial circulation. Jaques et al.<sup>10</sup> demonstrated the possibility of such clearance with subsequent detection of squamous cells in distal organs.

The results of this study have important clinical implications suggesting that detection of fetal squamous cells in significant numbers in blood aspirated from the distal port of a pulmonary arterial catheter is not diagnostic of amniotic fluid embolism. This perhaps explains, to some extent, the heterogeneity of clinical situations in which amniotic fluid embolism has been alleged to occur,<sup>13</sup> as well as the variety of clinical findings and hemodynamic aberrations commonly attributed to this single pathologic entity. The detection of squamous cells in the maternal pulmonary arterial circulation may be a necessary, but not sufficient, condition for the diagnosis of amniotic fluid embolism. In a critically ill obstetric patient, such a finding should not deter the clinician from a thorough search for other causes of hemodynamic instability (sepsis, cardiac disease, thromboembolism, etc.). Confirmatory studies may further contribute to our understanding of the phenomenon of amniotic fluid embolism.

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# Is there a causal connection between motile curved rods, *Mobiluncus* species, and bleeding complications?

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Motile curved rods, *Mobiluncus* species, were identified in 20 women whose main complaints were sparse vaginal bleeding and/or foul-smelling discharge. After treatment with metronidazole all the women were free of symptoms and no *Mobiluncus* could be detected. (AM J OBSTET GYNECOL 1986;154:107-8.)

**Key words:** *Mobiluncus*, metronidazole, bleeding complications

Thomason et al.<sup>1</sup> defined the presence of motile rods in wet smears: "The nature of motion is diagnostic, wave-like, rapid movements of short duration in a straight line or slightly arched direction, rarely moving out of the viewing field. Characteristic corkscrew or spinning activity is seen as one end of the bacteria appears to attach to the epithelial cell or to the glass slide; the bacteria then break away and move in a pathognomonic wave-like pattern before stopping again."<sup>1</sup> The clinical significance of *Mobiluncus* species is not yet established, although it has been associated with bacterial vaginosis.

The purpose of the present communication is to present 20 cases of *Mobiluncus* species and to point out some clinical implications such as foul-smelling discharge and bleeding complications and the effects of treatment with metronidazole.

## Material and methods

Women attending the outpatient clinic of the Department of Gynecology and Obstetrics at Central Hospital in Skövde, Sweden, because of foul-smelling discharge and/or bleeding complications were examined with wet-smear preparations. Women in whom the wet smears showed *Mobiluncus* species, by the definition of Thomason et al., were incorporated in this study. A careful medical, obstetric and gynecologic, and sexual history was taken, and special notes were made of any past or present complaints of vaginal discharge, pain, or spotting. A wet smear of vaginal secretion was performed with normal saline solution for observation by phase contrast microscopy with the use of the definition of *Mobiluncus* species described above. The presence of "clue cells" was noted. After one drop of 10% potassium hydroxide was mixed with the discharge specimen on a glass slide, the presence of a "fishy amine odor" was

noted ("sniff test"). The microscopic examinations were done by one investigator. Urethral and cervical swabs were placed in transport medium for incubation on McCoy cells for isolation of *Chlamydia trachomatis*. A vaginal swab was inoculated in Stuart's medium for isolation of *Gardnerella vaginalis* and *Mobiluncus* species.<sup>2</sup> After treatment all women underwent follow-up examination after 1 to 2 months.

## Results

In 14 women the main clinical complaints were sparse vaginal bleeding episodes or spotting lasting from 1 to 12 months. Thirteen of these women were treated with metronidazole, 500 mg three times a day for 10 days. At follow-up 1 to 2 months later the clinical symptoms had disappeared and no *Mobiluncus* species could be detected by wet-smear examination. These women were further followed up for 1 to 7 months without recurrences of their bleeding complications (Table I). In one woman treatment was withdrawn on the sixth day because of generalized exanthema. *Mobiluncus* organisms were still present at follow-up so she was given lymecycline. Discharge, lasting from 1 to 18 months, was the main symptom in six women. All 20 women fulfilled the criteria for the diagnosis of bacterial vaginosis. Metronidazole, 400 or 500 mg three times a day for 10 days was given to these women. At follow-up after 1 to 2 months the wet smears were normal and no complaints of discharge were noted. They were further followed up for 1 to 4 months with the same results (Table I).

To confirm the microscopic diagnosis of *Mobiluncus* species the bacteria were isolated in 10 cases by the method described by Holst et al.<sup>2</sup> *Chlamydia trachomatis* could not be isolated from any of the patients.

## Comment

The diagnosis of *Mobiluncus* species has been made either by isolation of the bacteria on special agar plates under anaerobic conditions<sup>2,3</sup> or by wet-smear preparations of vaginal discharge. The microscopic identi-

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Table I. Details of the patient material

Patient	Age (yr)	Clinical findings	Duration	Follow-up	Contraception
B. K.	38	Discharge	Years	2 mo	None
K. B.	26	Spotting	6 mo	6 mo	OC
A. F.	26	Spotting/discharge	4 mo	6 mo	IUD <sup>3</sup>
M. J.	40	Spotting	12 mo	3 mo	DMPA <sup>4</sup>
N. K.	23	Spotting	3 mo	4 mo	Mechanical
S. S.	37	Spotting/LPP	1 mo	2 mo	IUD
I. B.	21	Spotting	3 mo	7 mo	OC
A. L.	32	Discharge	18 mo	5 mo	IUD
U. H.	43	Discharge	2 mo	2 mo	Hysterectomy, 1981
K. L.	41	Discharge	1 mo	2 mo	Sterilized
M. P.	33	Spotting/discharge	1 mo	3 mo	Sterilized
Y. E.	42	Spotting	2 mo	2 mo*	IUD
A. L.	32	Spotting	2 mo	1 mo	Sterilized
A. M.	27	Spotting/discharge	2 mo	1 mo	OC
L. L.	49	Spotting	2 mo	3 mo	IUD
L. L.	19	Spotting/discharge	4 mo	2 mo	OC
C. J.	27	Spotting/LPP	2 mo	2 mo	IUD†
M. E.	40	Discharge	6 mo	1 mo	None
K. M.	31	Spotting	12 mo	1 mo	IUD
M. E.	44	Discharge	9 mo	2 mo	None

LPP = Lower pelvic pain; OC = oral contraceptives; IUD = intrauterine contraceptive devices; DMPA = depot medroxyprogesterone acetate.

\*Treatment was withdrawn on the sixth day because of generalized exanthema; *Mobiluncus* organisms were still present at follow-up so she was given lymecycline.

†No signs of salpingitis on laparoscopic examination.

fication of *Mobiluncus* species was quite typical by the definition of Thomason et al.<sup>1</sup>

*Gardnerella vaginalis* and recently *Mobiluncus* species have been associated with bacterial vaginosis.<sup>3</sup> Some authors assert that *Mobiluncus* species always appears together with *Gardnerella vaginalis*, but in our observations *Gardnerella vaginalis* could not be isolated from five of the patients.

Spotting has been associated with cervicitis and *Chlamydia trachomatis* is suggested as a possible agent. In the present material 13 women had spotting lasting for at least 1 month. *Chlamydia trachomatis* could not be isolated from any of them. In none of these women could *Mobiluncus* species be identified after treatment with metronidazole and the spotting episodes ceased. *Mobiluncus* species has not showed sensitivity to metronidazole in in vitro investigations. In the present clinical observation *Mobiluncus* species could not be identified at follow-up after treatment with metronidazole for 10 days. The explanation could be that *Mobiluncus*

species is sensitive to some of the metabolites of metronidazole. The occurrence of *Mobiluncus* species in association with bleeding complications presented in this communication is of great clinical interest, especially as there seems to be an effective treatment in the form of metronidazole. These observations have to be further investigated in a prospective controlled double-blind study.

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# Protease production by microorganisms associated with reproductive tract infection

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Factors influencing pathogenicity of various microbes found in the female lower genital tract remain incompletely understood. Protease production by cervico/vaginal microorganisms may alter or inactivate a variety of proteins important in host defense and structural-functional integrity including collagen-containing chorioamniotic membranes and uterine cervix. Host tissues may be made more susceptible to other organisms' virulence factors by protease-producing members of genital tract local flora. Microorganisms themselves may also be influenced by the presence of other microbial protease. Nonspecific protease, gelatinase, collagenase, and elastase production was examined for in vitro with use of aerobic (30) and anaerobic (25) strains of microorganisms typical of those isolated from the lower genital tract of women with premature rupture of membranes, chorioamnionitis, and puerperal infection. Microorganisms including *Bacteroides bivius*, *Bacteroides melaninogenicus*, *Bacteroides fragilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Proteus* species, and *Propionibacterium acnes* produce various proteases. Protease production by both acknowledged pathogenic and commensal bacteria may contribute to the occurrence of reproductive tract morbidity including premature rupture of membranes and preterm labor. (AM J OBSTET GYNECOL 1986;154:109-14.)

**Key words:** Protease, bacteria, pathogenesis, pelvic infections

Certain complications of pregnancy, such as premature rupture of membranes, chorioamnionitis, and even preterm labor and abortion, are frequently associated with histologic tissue findings of inflammation and microbiologic recovery of "normal microflora."<sup>1, 2</sup> The pathogenesis of such complications of pregnancy in the absence of anatomic or genetic abnormalities remains in large measure unexplained, and preventive strategies are limited. Surveys of genital tract flora in various states of genital tract health and disease frequently demonstrate potentially pathogenic organisms, such as *Staphylococcus aureus*, as well as less virulent "commensal" organisms such as *S. epidermidis*, *Propionibacterium acnes*, *Lactobacillus* species and a variety of anaerobic microorganisms.<sup>3</sup> Bacterial inhabitants of ecologic "niches" within the female genital tract are dynamic and vary in number and composition with the host's hormonal status.<sup>3</sup> Complex interrelationships between microorganisms among themselves and with host tissues are increasingly understood as are physiologic details of local microbial environments.<sup>4</sup> Similarly, the

pathogenesis of various infections is being increasingly defined with the development of methods able to demonstrate potential and proven microbial virulence factors. Study of possible virulence factors arising in genital tract microflora may allow for increased understanding of pathogenesis and possible prevention and treatment of "idiopathic" premature rupture of membranes and preterm labor.

Collagen, elastin, and other structural proteins are important for the physical integrity and function of the reproductive tract, especially during the uterine and cervical alterations brought about by pregnancy, parturition, and the puerperium.<sup>5</sup> Since collagenases, elastases, and other proteases play dynamic roles in disease at other body sites, we examined microorganisms frequently found in the female genital tract for collagenase, elastase, and nonspecific protease activity.

## Material and methods

Test microorganisms were collected from women with prematurely ruptured membranes, preterm labor, or puerperal infection. Identifications were performed with use of standard aerobic and anaerobic microbiologic methodology.<sup>6</sup> Whenever representative samples of microorganisms described in studies of genital tract microbiology could not be obtained, University of Colorado Health Sciences Center Microbiology Laboratory and/or American Type Culture Collection isolates were

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Table I. Isolates tested in aerobic conditions\*

	No. of isolates	Horsehide blue hydrolysis	Martley's buffered caseinate agar	Gelatinase	Collagenase	Elastinase
<i>B. melitensis</i>	1	1/1	NG	—	1/1	NG
<i>B. suis</i>	1	0/1	NG	1/1	NG	NG
<i>C. albicans</i>	5	1/5	—	—	—	—
<i>Corynebacterium</i> sp.	15	0/4	NG	1/4	NG	NG
<i>E. coli</i>	8	2/8	—	—	1/8	—
<i>G. vaginalis</i>	7	1/2	NG	2/7	NG	NG
<i>H. influenzae</i> , type b	3	0/3†	NG	2/3	NG	NG
<i>K. pneumoniae</i>	1	0/1	1/1	—	—	—
<i>L. casei</i>	1	0/1	—	—	—	NG
<i>L. monocytogenes</i>	22	21/22	—	—	—	—
<i>M. morgani</i>	3	3/3	2/3	—	—	—
<i>N. gonorrhoeae</i>	8	5/8	NG	1/7	—	NG
<i>P. aeruginosa</i>	6	6/6‡	6/6	6/6	3/6	3/6
<i>P. mirabilis</i>	5	5/5‡	4/5	3/5	2/5	—
<i>P. vulgaris</i>	2	2/2	2/2	1/2	—	—
<i>S. aureus</i>	3	3/3	3/3	3/3	3/3	2/3
<i>S. epidermidis</i>	15	15/15‡	14/15	6/15	5/15	4/15
<i>S. saprophyticus</i>	2	—	1/2	—	—	—
<i>S. pyogenes</i> (group A streptococcus)	3	3/3	—	—	—	—
<i>S. agalactiae</i>	5	1/5	—	—	—	—
<i>S. faecalis</i>	2	—	1/2	—	—	—
<i>S. mitis</i>	1	—	—	—	—	NG
<i>S. mutans</i>	1	—	—	—	—	NG
<i>S. pneumoniae</i>	1	—	—	1/1	—	NG
Group D streptococcus (enterococcus)	1	1/1	1/1	1/1	—	—
Yeast sp.	1	—	—	1/1	—	—
Nonhemolytic streptococcus	1	—	1/1	—	—	1/1
Total	124					

NG, No growth. —, No activity.

\*37° C, 10% carbon dioxide.

†Markedly less active than controls.

‡Markedly more active than controls.

used to furnish a more complete sampling. Tables I and II list the identification of 27 aerobic and 27 anaerobic groups of microorganisms evaluated. Since each group contained up to 22 organisms, a total of 302 isolates were evaluated. As both microbial cell-associated and extracellular microbial proteases may affect reproductive tract tissues, test organisms were assayed for protease activity in systems which detected both cell-associated and extracellular proteases in media systems approximating ranges of pH and pCO<sub>2</sub> encountered in lower female genital tract environments and known to enhance protease production.<sup>7</sup> Pre-reduced, supplemented peptone broth (Supplemented Peptone Broth II, Becton-Dickinson, Paramus, New Jersey) was used as liquid media throughout. The selection of a standard liquid media excluded evaluation of mycoplasmas, protozoas, *Chlamydia trachomatis*, and other organisms. Aerobic test organisms were incubated at 37° C in 10% carbon dioxide with agitation at 250 rpm (gyratory shaker model No. G2, New Brunswick Scientific Company, Edison, New Jersey). Anaerobic incubations were performed in Gas Pack contain-

ers at 37° C without agitation. Cultures were maintained in 25% glycerol at -80° C and recultured on appropriate agar before use. Purity of test cultures was confirmed by duplicate cultures on appropriate media. Cultures were routinely checked for contamination. Each organism was tested in triplicate.

**Nonspecific protease determination.** With use of the method of Rinderknecht et al., proteolytic activity of test organisms in supplemented peptone broth was evaluated in situ with use of 4 mg of horsehide blue (Sigma Chemical Company, St. Louis) in 1 ml of standard media in which test organisms were inoculated to achieve a starting standard optical density of 0.500 at 590 nm.<sup>8</sup> Aerobic microorganisms were grown for 10 hours in 10% carbon dioxide at 37° C while being agitated at 250 rpm. Anaerobic and slower-growing aerobic organisms were incubated until visible growth occurred, usually by 5 days. Anaerobic organisms were inoculated in peptone media and incubated in a Gas Pak at 37° C. All samples were compared with duplicate uninoculated but similarly treated control mixtures of media and substrate. At the end of incubation the

**Table II.** Isolates tested in anaerobic conditions\*

	No. of isolates	Horsehide blue hydrolysis	Martley's buffered caseinate agar	Gelatinase	Collagenase
<i>A. israelii</i>	1	—	NG	—	—
<i>A. odontolyticus</i>	3	—	NG	—	—
<i>B. asaccharolyticus</i>	2	1/2	2/2	1/2	—
<i>B. bivius</i>	5	5/5	5/5	3/4	4/5
<i>B. distasonis</i>	5	5/5	NG	—	2/5
<i>B. fragilis</i>	12	4/12	NG	—	—
<i>B. melaninogenicus</i>	8	1/6	4/4	—	2/8
<i>B. malnogenicus</i> — <i>asaccharolyticus</i>	2	2/2	1/2	—	—
<i>B. ovatus</i>	2	—	1/1	—	—
<i>B. ruminicola</i>	1	1/1	1/1	—	—
<i>B. thetaiotaomicron</i>	5	5/5	3/5	—	1/5
<i>B. uniformis</i>	1	0/1	—	—	—
<i>B. ureolyticus</i>	1	1/1	1/1	1/1	—
<i>B. vulgatus</i>	2	—	—	—	—
<i>C. perfringens</i>	5	2/5	5/5	5/5	5/5
<i>E. corrodens</i>	4	4/4	NG	—	—
<i>F. mortiferum</i>	1	1/1†	NG	—	—
<i>F. nucleatum</i>	2	2/2	NG	—	1/2
<i>Peptococcus</i> sp.	5	0/5	1/5	1/5	2/2
<i>P. anaerobius</i>	1	—	—	1/1	—
<i>P. magnus</i>	1	1/1	—	1/1	—
<i>P. asaccharolyticus</i>	4	0/4	4/4	—	3/3
<i>Peptostreptococcus anaerobius</i>	1	1/1	NG	—	1/1
<i>P. asaccharolyticus</i>	1	—	NG	—	—
<i>P. acnes</i>	5	5/5	5/5	5/5	—
<i>V. parvula</i>	2	0/2	—	—	—
Miscellaneous anaerobes	4	1/4	—	—	—
Total	86				

\*Gas Pak, 37° C.

†Markedly less active than control samples.

horsehide blue-containing cultures were centrifuged at  $1000 \times g$  for 15 minutes and the amount of solubilized dye released by protein hydrolysis measured colorimetrically at 595 nm. Individual organisms were run in triplicate and were considered positive if the mean value of the triplicates of each organism exceeded 100% of control measurements. Control values were less than  $\Delta 595 = 0.1$ , while variation between triplicates was less than 10%. *S. aureus* strain 581 served as the control protease producer with a mean  $\Delta 595$  value of  $0.85 \pm 0.06$ .

**Martley modified caseinate agar.** Martley's modified caseinate agar, also known as standard methods casein agar, was prepared as described elsewhere.<sup>9</sup> Test cultures and control aliquots of uninoculated media were inoculated onto standard methods casein agar plates which were incubated aerobically (10% carbon dioxide at 37° C) or anaerobically (Gas Pak at 37° C). Test plates were inspected daily. *Proteus* species were inoculated on individual standard methods casein agar plates and incubated within individually sealed plastic bags at 37° C in 10% carbon dioxide (Figs. 1 and 2). Media clearing and/or immunoprecipitation-like rings indicative of protease production were observed.<sup>9</sup> Positive control for protease production was *S. aureus* strain 581.

**Elastase.** Agar plate assays for elastase activity were prepared by the method of Sbarra<sup>10</sup> with use of a 1% sterile elastin (Sigma Chemical) in Noble agar (Difco Laboratories, Detroit). Plates were inoculated and incubated aerobically or anaerobically as appropriate with each plate placed in a sealed plastic bag and observed for 1 week. *Proteus* species were placed on individual plates. Clearing of elastin substrate media surrounding or underlying test isolate colonies was interpreted as demonstrating elastase activity.<sup>10</sup> The positive elastase control was hog pancreatic elastase, type 1 (Sigma Chemical). One unit of elastase gave a zone diameter of  $0.8 \text{ cm} \pm 0.03$ . Individual organisms were plated in triplicate.

**Gelatinase.** Gelatinase activity was evaluated by inoculating test microorganisms into 1 ml of supplemented peptone broth to achieve a starting inoculum density of optical density 0.500 at 595 nm. Aerobic isolates were incubated for 10 hours with agitation at 250 rpm at 37° C in 10% carbon dioxide before  $1 \times 0.25 \text{ cm}$  strips of sterile gelatin-coated roentgenographic film were placed on end in each aliquot of incubated media and uninoculated control broth aliquots without further agitation. Slow-growing aerobes and anaerobic organisms were incubated as previously described until visible growth occurred, at which time



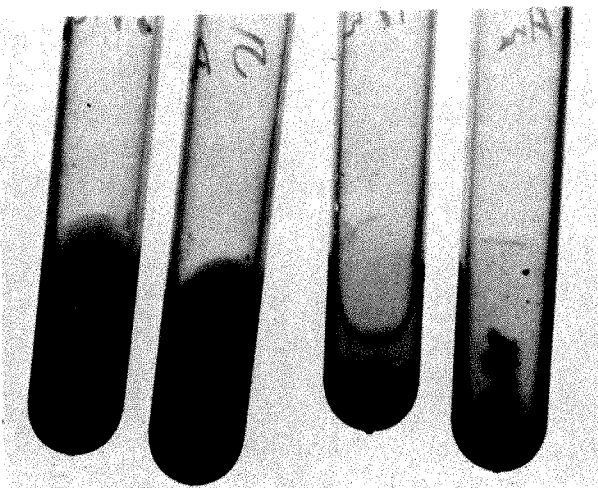


Fig. 1. Quantitative release of dye with horsehide blue hydrolysis measured by colorimeter.

gelatin test strips were placed into the media. Complete clearing of gelatin coating at 10 hours of incubation was considered evidence of gelatinase production. Each organism was run in triplicate.

**Collagenase.** Collagenase activity was determined with use of the thin collagen film method of Levenson.<sup>11</sup> A film of purified collagen (Sigma Chemical) was applied to Noble agar (Difco) coated glass plates. Test organisms prepared as described for gelatinase was placed in 20  $\mu$ l aliquots on level collagen film plates. These stationary plates were incubated at 37° C in 10% carbon dioxide within a sealed humidified chamber for 10 hours and then rinsed gently with tap water and counterstained for 4 minutes with 0.5% Coomassie blue in 10% acetic acid and air dried. The presence of collagenase was determined by the presence of a clear collagen-free zone at the site of inoculation against a field of blue-stained collagen film. The positive control for collagenase was *C. histolyticum* collagenase, type III (Sigma Chemical). One unit of collagenase yielded a clearing diameter of 0.4 cm  $\pm$  0.05.

## Results

We have described in vitro production of a variety of proteases produced by a number of microorganisms recoverable from the lower genital tract in health and disease. Proteases were noted to be produced extracellularly as determined by both the Martley and elastin agar plate assays. Extracellular and/or cell-associated protease production was indicated by testing with horsehide blue, collagenase, and gelatinase tests performed in broth. Production of proteases by several microorganisms, including *Bacteroides melaninogenicus* and *B. asaccharolyticus*, has been previously described in restricted numbers of oral and other body site isolates.<sup>12</sup> We confirm protease production in specimens of these

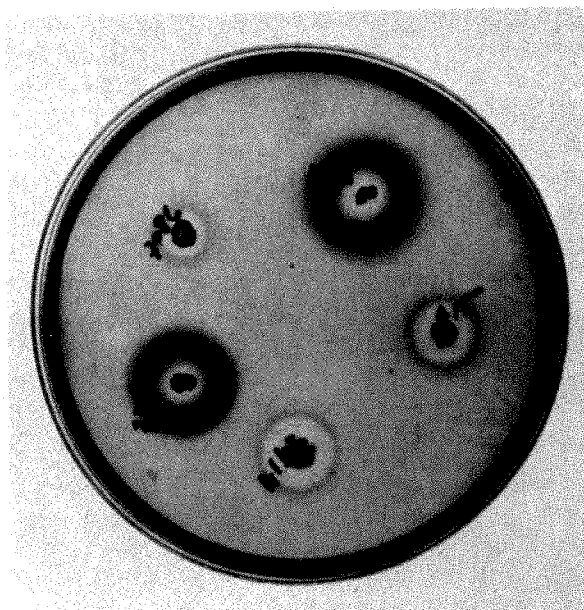


Fig. 2. Martley's (caseinate) agar demonstrating effect of proteases released by specimen's growth.

organisms isolated from genital tract and other body sites. Aerobic microorganisms previously known to produce protease(s) include *S. aureus*, group A *Streptococcus*, as well as various *Pseudomonas* and *Proteus* species which also produced protease(s) in our test systems.<sup>13</sup> Heterogeneity of test results within species groups occurred and has been noted in prior studies with multiple strains of the same microorganisms evaluated with gel electrophoresis and specified enzyme substrate tests.<sup>13</sup> Only two of five isolates of *Clostridium perfringens* demonstrated proteolytic activity with horsehide blue. Possible explanations for these observations include genetic heterogeneity, autodegradation of protease(s), or the presence of protease inhibitors.

Organisms not previously systematically examined for protease production but demonstrating marked activity include prominently *B. bivius*, *S. epidermidis*, and *P. acnes*. Multiple isolates of *B. bivius*, like many other anaerobes, have not been examined for protease production by microbial taxonomists. That these and other nonspecific protease-producing microorganisms may constitute "normal" lower genital tract microflora and are frequently recovered from the lower genital tract in situations such as preterm labor, premature rupture of membranes, puerperal infections, and acute salpingitis suggests possible additive or synergistic roles for these organisms in overt and possibly subclinical reproductive tract infection. More specific characterization of the proteases produced by these organisms awaits detailed investigation, as does demonstration of clinical relevance.

Microorganisms capable of producing collagenase

prominently include *S. aureus*, *P. aeruginosa*, *P. vulgaris*, as well as *Brucella melitensis*, *B. asaccharolyticus*, *B. bivius*, *C. perfringens* (Tables I and II). Examination of elastase production was limited because test medium was unable to support growth of many organisms, including most anaerobic test isolates. Because of this paucity of growth, anaerobic results for elastase are not reported. *S. aureus* and *S. epidermidis*, along with *P. aeruginosa*, all demonstrated elastase activity.

Protease production by limited number of strains of group III *S. agalactiae* has been demonstrated.<sup>14</sup> In our studies, *S. agalactiae* lacked collagenase, elastase, caseinase, and gelatinase activity. Only one of five untyped isolates was positive for production of nonspecific protease. This suggests that *S. agalactiae* likely possesses other virulence factors (hemolysin, hyaluronidase, nuclease, etc.), which may account for this organism's prominence in perinatal sepsis.

We have demonstrated that lower genital tract bacteria frequently considered as commensals (such as *S. epidermidis* and *P. acnes*) and microorganisms of acknowledged virulence (such as *S. aureus*, *Listeria monocytogenes*, *Pseudomonas* and *Proteus* species), as well as an indeterminate virulence (such as *B. bivius*, *B. melaninogenicus*, and *B. thetaiotaomicron*), produce a variety of proteases which are of possible significance in the pathogenesis of reproductive tract disease.

Production of specific proteases may contribute to disease processes in a variety of ways. Lower genital tract microorganisms occur within the lower uterine segment and amniotic fluid at term with some frequency.<sup>15</sup> Microbial proteases, such as collagenase and elastase, released into the genital tract milieu may act to structurally damage connective tissue in the cervix and chorioamniotic membranes, possibly without overt signs of infection. Microbial proteases may also saturate local antiprotease substances, such as  $\alpha$ -2-macroglobulin, allowing increased activity of unblocked endogenous tissue collagenases.<sup>16</sup> Various bacterial proteases may act as chemotactic factors that elicit host inflammatory cell migration with subsequent local tissue release of neutrophil or macrophage lysosomal collagenases and elastinase if degranulation occurs. Protease from one species or organisms may "activate" zymogen or proenzymes produced by other microorganisms as host cells. Further, protease-producing microorganisms may influence the growth of other microbes by supplying growth factors from host or other microbial sources. An example may be *Gardnerella vaginalis*' suggested enhanced growth in the milieu produced by metabolic activities of various anaerobic microorganisms.<sup>17</sup> Production of specific proteases may therefore be a complex but decisive virulence factor in the setting of both monoetiologic or synergistic infection.

If the relevancy of protease production by lower genital

reproductive tract microorganisms can be shown by clinical correlation and experimentation with in vivo and in vitro models of infection, then consideration may be given to possible local inhibition of microbial proteases. In animal studies, Brock et al.<sup>18</sup> demonstrated that rumen bacteria contain serine, cysteine, and metalloprotein proteases that can be selectively inhibited by synthetic, plant, or microbial protease inhibitors. Systemic administration of a serine protease inhibitor, gabexate mesilate (FOY), appears to block protease activity in models of shock.<sup>19</sup> Subinhibitory concentrations of lincomycin-derived antimicrobials reduce extracellular protease production by group A streptococcus and *S. aureus* and lipase production by *Propionibacteria* species.<sup>20, 21</sup> Subminimal inhibitory concentration levels of vancomycin inhibit cytotoxin production by *C. difficile* by altering cell wall release mechanisms.<sup>22</sup> Reduced production of multiple extracellular proteases appears as a function of clindamycin's effect on peripherally localized bacterial ribosomes.<sup>23</sup> Inhibition of microbial protease production may be an unrecognized attribute of antimicrobial treatment. In vitro and in vivo models establishing pathogenic effects of specific bacterial proteases on reproductive tract function are required before treatment schemes can be evaluated.

### Comment

Factors influencing pathogenicity of various microbes singly or in conjunction with other microorganisms found in the lower genital tract remain incompletely understood. Extracellular protease production constitutes a potential factor that may alter or inactivate a variety of host proteins including surface antimicrobial factors and collagenous structures. Adjacent microorganisms may also be influenced by protease production, allowing for additive or synergistic activity. Protease production was examined for isolates of aerobic (124) and anaerobic (86) representative strains of microorganisms found in the lower and upper genital tract sites of women with premature rupture of membranes and chorioamnionitis. *B. bivius*, *B. melaninogenicus*, *B. fragilis*, *P. aeruginosa*, *E. coli*, group A streptococcus, and various streptococcal, staphylococcal, and *Proteus* species, among others, produce proteases. Production of specific proteases may correlate with pathogenicity of genital tract microorganisms that are otherwise considered "normal flora." Proof of the relevancy of these findings requires clinical correlation and further experimentation.

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# Human papillomavirus deoxyribonucleic acid in cervical carcinoma from primary and metastatic sites

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Tissue from 13 cervical cancers and pelvic or para-aortic lymph nodes from the same patient were evaluated by deoxyribonucleic acid hybridization with a human papillomavirus type 16 deoxyribonucleic acid probe for the presence of human papillomavirus-related deoxyribonucleic acid sequences. Twelve of the primary malignancies were squamous cancers and one was an adenocarcinoma. Eight of the primary tumors contained human papillomavirus type 16 deoxyribonucleic acid sequences, and five contained viral sequences closely related to human papillomavirus type 16. Histopathologic diagnosis confirmed malignant cells in six of 13 lymph nodes; three of these specimens contained human papillomavirus type 16 sequences while three had human papillomavirus type 16-related sequences. One lymph node that failed to show malignant cells also contained human papillomavirus type 16 deoxyribonucleic acid. The remaining lymph nodes did not contain malignant cells by either histologic examination or deoxyribonucleic acid hybridization. The human papillomavirus deoxyribonucleic acid sequences in the lymph nodes were similar to those in the matched primary cancer in all cases. These data provide further evidence implicating human papillomavirus in the etiology of cervical cancer. (AM J OBSTET GYNECOL 1986;154:115-9.)

**Key words:** Human papillomavirus, cervical cancer, metastases

Recently, attention has focused on the role of human papillomavirus (HPV) in cervical neoplasia. This association is based on morphologic, immunocytochemical, and molecular hybridization data showing evidence of HPV in the vast majority of intraepithelial and invasive neoplasms. Morphologic changes consistent with HPV infection can be recognized in 80% of cervical intraepithelial neoplasia,<sup>1</sup> HPV structural antigens are present in nearly 50% of mild and moderate dysplasia,<sup>2,3</sup> and molecular hybridization studies have revealed HPV deoxyribonucleic acid (DNA) sequences in 73% of all grades of dysplasia.<sup>4</sup> Biopsy specimens of cervical cancers have also been shown to contain HPV DNA sequences. Gissmann et al.<sup>5</sup> first demonstrated HPV-11 DNA sequences in five of 27 cervical cancers (three invasive and two carcinoma in situ). The genome of another virus, HPV-16, which was obtained from a biopsy specimen of invasive cervical cancer, has been shown to be present in 19 of 41 (46%) cervical cancers from German, African, and Brazilian patients.<sup>6</sup> In ad-

dition, DNA of yet another virus, HPV-18, has been molecularly cloned from a cervical cancer and these sequences are present in about 22% (11 of 51) of cervical cancer biopsy specimens from women in Germany, Africa, and Brazil.<sup>7</sup> However, when nonstringent hybridization techniques were used, HPV DNA sequences only distantly related to HPV-16 and HPV-18 could be detected in an additional 14% of cervical cancers. Thus approximately 80% of both intraepithelial and invasive cervical neoplasms have been shown to contain HPV DNA sequences.

Since HPV sequences are detectable in biopsy specimens of dysplasia, it is possible that the viral DNA detected in invasive cancers is from adjacent intraepithelial lesions and therefore represents contamination. To clarify this potential problem in the interpretation of molecular hybridization studies, we performed molecular hybridization analysis on DNA extracted from the primary tumor and pelvic or para-aortic lymph nodes from 13 patients with advanced stage cervical cancer. The results indicate that HPV sequences present in biopsy specimens of invasive cervical cancer are not the result of contamination from cervical dysplasia since the identical viral DNA sequences are present in DNA extracted from lymph nodes containing metastatic cervical cancer in the same patient.

## Material and methods

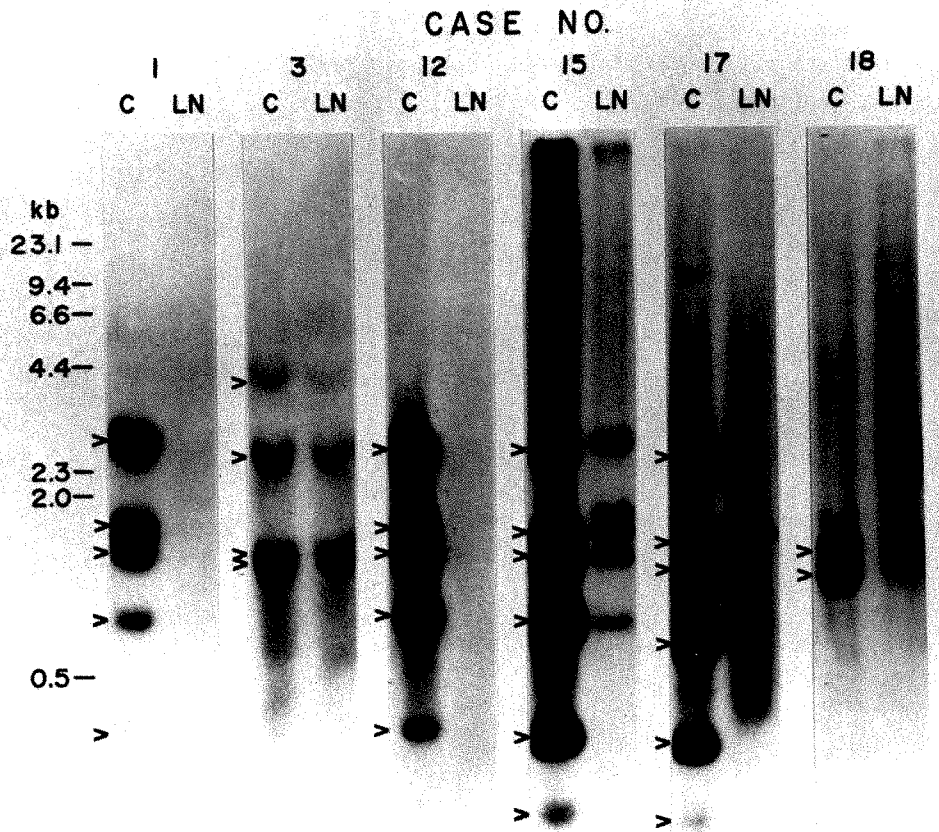
**Clinical material.** Tissue specimens were obtained from Peruvian women seen at an oncology clinic for abnormal Papanicolaou smears. The patients ranged

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**Fig. 1.** Southern blot hybridization of HPV-16 probe DNA to DNA isolated from cervical (C) or lymph node (LN) biopsy specimens. Cellular DNA was digested with *Pst*I and approximately equal quantities (10  $\mu$ g) electrophoresed in parallel and probed for HPV sequences. Virus-specific DNA sequences in the cervical biopsy lanes (C) are indicated by arrows and corresponding sequences are present in lymph node biopsy lanes (LN) for Cases 3, 15, and 18. The restriction enzyme cleavage pattern of Case 1 is identical to the prototype HPV-16 described by Dürst et al.<sup>6</sup> Lymph node DNA samples for Cases 1, 12, and 17 did not contain detectable HPV DNA sequences. The size marker (in kilobases) was *Hind*III-digested bacteriophage lambda DNA. Autoradiogram exposures were for 48 hours at  $-70^{\circ}$  C in the presence of intensifying screens.

in age from 24 to 63 years with a mean of 42.3 years. The diagnosis of cervical cancer was made on biopsy specimens of the cervix. All patients had clinical and radiologic staging procedures and thereafter an exploratory laparotomy with selected sampling of pelvic or para-aortic lymph nodes.<sup>8</sup> All patients had Stage IIB or greater disease.

**Preparation of tissue samples.** The preoperative biopsy specimen of cervical tumor was bisected; one portion was fixed in formalin for histologic examination and the portion stored at  $-70^{\circ}$  C. Clinically questionable lymph nodes were bisected and treated in the same fashion as the cervical biopsy specimen. The tissues were snap-frozen, mounted in water, and sectioned on a cryostat. A representative section from the specimen was stained with hematoxylin and eosin for histologic examination. Total cellular DNA was extracted from the remaining sections for molecular hybridization as previously described.<sup>4</sup>

**Preparation of radiolabeled HPV-16 DNA.** HPV-16 DNA cloned into the bacterial plasmid vector pBR322 was provided by L. Gissmann. Virus DNA was released from flanking plasmid DNA sequences by *Bam*HI restriction enzyme cleavage and the viral sequences electroeluted from agarose gels. DNA was labeled by nick translation with *Escherichia coli* DNA polymerase and deoxyadenosine triphosphate labeled with radioactive phosphorus to a specific activity of  $1$  to  $2 \times 10^8$  cpm per microgram of DNA.<sup>9</sup>

**Blot transfer hybridization.** Cellular DNA was digested with a fourfold excess of the restriction endonuclease *Pst*I for 4 hours at  $37^{\circ}$  C and electrophoresed through 0.8% agarose gels. The DNA was transferred to cellulose nitrate membranes essentially as described by Southern.<sup>10</sup> After the membranes were baked in a vacuum at  $80^{\circ}$  C for 2 hours, they were incubated in a solution containing 0.6 mol/L sodium chloride and 0.06 mol/L sodium citrate in  $10\times$  Denhardt's<sup>11</sup> solution

**Table I.** Frozen-section diagnoses and HPV-16 DNA hybridizations to DNA extracted from cervical cancers and lymph nodes from the same patient

Case No.	Cervix		Lymph node	
	Frozen section	HPV DNA	Frozen section	HPV DNA
1	Squamous carcinoma	HPV-16	No tumor*	Negative
3	Squamous carcinoma	HPV-31	Squamous carcinoma	HPV-31
5	Adenocarcinoma	HPV-A†	Adenocarcinoma	HPV-A
6	Squamous carcinoma	HPV-31	No tumor	Negative
7	Squamous carcinoma	HPV-16	Squamous carcinoma	HPV-16
8	Squamous carcinoma	HPV-B	No tumor	Negative
9	Squamous carcinoma	HPV-16	No tumor	Negative
10	Squamous carcinoma	HPV-16	No tumor	HPV-16
11	Squamous carcinoma	HPV-16	No tumor*	HPV-16
12	Squamous carcinoma	HPV-16	No tumor	Negative
15	No tumor*	HPV-16	Squamous carcinoma	HPV-16
17	Squamous carcinoma	HPV-16	No tumor	Negative
18	Squamous carcinoma	HPV-C	Squamous carcinoma	HPV-C

\*Squamous cell carcinoma was present in the formalin-fixed specimens in these cases.

†HPV-A, HPV-B, and HPV-C represent viral DNA that exhibits *Pst*I cleavage patterns different from the prototype HPV-16 pattern (Fig. 1, Case 1).

(0.2% each Ficoll polyvinylpyrrolidone, and bovine serum albumin) with 50 µg/ml of sonicated and denatured human lymphocyte DNA. Hybridizations were carried out in a solution containing 1 to 5 × 10<sup>6</sup> cpm of a heat-denatured probe, 1 mol/L sodium chloride, 0.15 mol/L tris(hydroxymethyl)-2-aminoethanesulfonic acid, pH 7.5, 40% formamide, and 2× Denhardt's solution with 50 µg/ml of sonicated, heat-denatured human lymphocyte DNA at 37° C for 24 hours. The membranes were washed extensively in 0.3 mol/L sodium chloride and 0.03 mol/L sodium citrate at 60° C and exposed to x-ray film for autoradiography. The conditions of hybridization were equivalent to 32° C below the melting temperature of the DNA (about 22% allowable base mismatch).<sup>12</sup>

## Results

**Histopathology.** Histologic examination of the portions of the 13 cervical biopsy specimens fixed in formalin revealed squamous carcinoma in 12 of the cases and adenocarcinoma in one. Frozen-section diagnosis of the cervical tissue used for molecular hybridization revealed invasive carcinoma in all cases except one (Case 15), in which only normal squamous epithelium was present. Histologic examination of the formalin-fixed lymph nodes revealed squamous carcinoma in six, adenocarcinoma in one, and no tumor in the remaining six cases. Microscopic examination of the frozen tissue used for molecular hybridization was concordant in 11 instances. In the remaining two cases, tumor was not identified in the frozen tissue but was present in the formalin-fixed specimens (Cases 1 and 11).

**Molecular hybridization.** With the use of an HPV-16 probe under moderately stringent conditions, HPV DNA sequences were detected in all of the primary

cervical cancers and in seven lymph nodes. Representative examples of the hybridization reactions are shown in Fig. 1.

Analysis of the cleavage products of the viral sequences in the cervical cancer biopsy specimens indicated that eight of 13 were similar or identical to the *Pst*I digestion pattern of the prototype HPV-16 described by Dürst et al.<sup>6</sup> Two cancers contained sequences that had fragments in common with HPV-16 and may have lost some *Pst*I recognition sequences (HPV-A and HPV-B, data not shown). Viral sequences in two of the cervical biopsy specimens (represented by Case 3, Fig. 1) contained a restriction pattern representative of a new HPV type (HPV-31) that has a limited amount of sequence homology to the HPV-16 probe (Lorincz A, et al., unpublished data). One biopsy specimen (Case 18) contained sequences with only limited homology to HPV-16 (HPV-C). The sum of the sizes of the restriction fragments of this HPV genome is significantly less than expected for papillomavirus DNA, suggesting that additional sequences may be present that have a low degree of homology to the HPV-16 probe under the conditions of hybridization employed. Thus these viral sequences may represent a new HPV type.

Variations in the sizes of some of the restriction fragments of HPV-16-containing lesions were also observed. In many instances extra bands were detected that could represent either incomplete restriction enzyme digestion or virus-cell DNA junction fragments resulting from integration. Although the lanes in the autoradiogram in Fig. 1 contained approximately equal quantities of cell DNA, there was considerable variation in the amount of HPV-16 detected almost to the point of obliteration of the signal (compare Case 1 with Cases



12, 15, and 17). Similar variations in viral genome copy number have been noted in other papillomavirus-induced tumors and transformed cells.<sup>13</sup>

**Correlation of histopathologic examination and molecular hybridization.** The results of histopathologic findings and DNA hybridization reactions are summarized in Table I. Of the seven lymph nodes shown to be positive for HPV DNA sequences, five contained metastatic cancer upon frozen-section evaluation. Two lymph nodes that contained HPV DNA sequences failed to reveal cancer cells in the frozen section used for histopathologic examination; however, one of these lymph nodes was shown to contain cancer cells in the permanent sections. Thus HPV DNA sequences were detected in each lymph node shown to contain cancer cells by histopathologic examination. In addition, the viral DNA present in each of the seven cases with a lymph node containing HPV DNA had restriction patterns similar to the viral sequences in the cervical cancer from the same patient.

There was no correlation between the presence of metastases and virus type (or a particular *Pst*I cleavage pattern) nor was there an association between viral sequences, clinical stage of disease, or histologic type. All of the cancers in this study, however, contained either HPV-16 sequences or HPV sequences that exhibited homology to the HPV-16 probe.

### Comment

All 13 cervical carcinomas examined in this study contained HPV DNA sequences. Likewise, HPV DNA sequences were detected in lymph nodes from six cases containing metastatic tumor. The viral sequences had the same restriction enzyme cleavage pattern as those in the primary cervical cancers. This is significant since four different cleavage patterns were represented among this group of matched primary and metastatic cancers. Moreover, all of the viral DNA found in the lesions represented sequences closely related to or identical to HPV-16, one of the virus types preferentially associated with cervical cancer.<sup>6</sup> In one case the lymph node sample contained HPV sequences but metastatic tumor was not identified. Since only a limited number of frozen sections can be evaluated from tissue from which DNA is extracted, it is likely that sampling error could account for this discrepancy. Similarly, the frozen-section evaluation of one of the cervical cancers showed normal squamous epithelium although HPV DNA sequences were readily detectable in the tissue DNA preparation and tumor was present in the formalin-fixed specimen.

There are several possible explanations for the finding of HPV DNA sequences in lymph nodes of patients with cervical cancer. First, the lymph nodes may contain intact HPV filtered during viremia. This is unlikely

since lymph nodes with normal histologic features in five patients failed to show HPV sequences and infectious virus has never been detected by immunologic techniques either in the serum or in the primary cervical lesion (Jenson AB, et al., unpublished results). Second, viral sequences, either free or cell-associated, could reach lymph nodes by lymphatic drainage from the site of the carcinoma. This too is unlikely since all but one of the cases with HPV DNA sequences in the lymph nodes occurred in nodes that contained histologic evidence of metastases. Furthermore, free DNA sequences would probably be degraded by enzymes released by necrotic cancer cells. The most likely explanation for the presence of HPV DNA in lymph nodes containing metastatic tumor is that the HPV DNA sequences are present in the tumor cells themselves. This conclusion is supported by the concordance of the molecular hybridization and histopathologic data.

There is precedence for the association of HPV DNA sequences in primary and metastatic lesions of squamous carcinomas arising in humans. This has been reported in patients with epidermodysplasia verruciformis, a rare autosomal recessive disease characterized by varying degrees of decreased cell-mediated immunity and increased susceptibility to HPV infection.<sup>14</sup> In approximately 25% of patients with epidermodysplasia verruciformis, malignant conversion occurs in pityriasis rosea-like warts exposed to sunlight. These lesions progress through increasing degrees of dysplasia, histologically similar to cervical dysplasias, before developing into carcinoma and eventually invasive cancer. These tumors may occasionally metastasize. Of the numerous HPV types preferentially associated with epidermodysplasia verruciformis, HPV-5 was identified in both primary and metastatic cancers of a patient with this disease.<sup>15</sup> Thus it appears that the role of HPV-5 in dysplastic and neoplastic lesions of epidermodysplasia verruciformis may be analogous to the role of HPV-16 and related HPVs in cervical carcinomas with distant metastases.

In this study as well as previous studies demonstrating HPV DNA sequences in cervical cancer biopsies, the possibility that viral sequences were derived from adjacent premalignant lesions (dysplasias) cannot be ruled out. Our finding of similar HPV DNA sequences in lymph nodes with metastatic carcinoma as well as the primary cervical tumor rules out this possibility and is the most compelling evidence to date implicating HPV in the pathogenesis of cervical cancer.

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# Vaginal colonization with *Escherichia coli* in healthy women

## Determination of relative risks by quantitative culture and multivariate statistical analysis

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The rate of vaginal colonization with *Escherichia coli* in 495 healthy women was 12% in a prospective study with use of selective media and semiquantitative culture techniques. Computer-assisted multivariate analysis revealed that vaginal *E. coli* was significantly associated with the menstrual phase of the cycle, prior use of antibiotics, use of diaphragm or cervical cap for contraception, history of previous urinary tract infection, and coisolation of *Staphylococcus aureus* that was positive for the toxic shock syndrome toxin-1 ( $p < 0.05$ , multiple stepwise logistic regression analysis). No significant association was observed with tampon use or brand, other contraceptive methods, sexual activity, genital symptoms, recent vaginal infection, or other personal habits. Quantitative cultures obtained sequentially throughout the menstrual cycle in 12 unselected women confirmed higher *E. coli* counts in menstrual or midcycle samples compared to paired premenstrual specimens ( $p < 0.05$ , Wilcoxon paired rank sign test). These data emphasize the hormonal and other host determinants in vaginal colonization by *E. coli* and may explain the high rate of vaginal *E. coli* (64%), in addition to toxicogenic *S. aureus*, in acute toxic shock syndrome and the higher incidence of urinary tract infection in women with diaphragm or cervical cap for contraception compared to other contraceptive methods. (AM J OBSTET GYNECOL 1986;154:120-6.)

**Key words:** Vaginal flora, *Escherichia coli*, menstrual cycle, contraception, urinary tract infection

The pattern of vaginal colonization with *Escherichia coli* in healthy young adults has not been well characterized. Vaginal, cervical, and periurethral Enterobacteriaceae, principally *E. coli*, have been of interest primarily because of their implication as opportunistic pathogens in various female genital tract infections and in the pathogenesis of recurrent urinary tract infection. Previous studies found a vaginal colonization rate of *E. coli* varying from 6% to 26%.<sup>1-3</sup> These data, largely based on qualitative cultures obtained from asymptomatic premenopausal women at a single point in time, often failed to consider the confounding influence of hormonal or other host factors on the colonization rate. In an earlier and preliminary report,<sup>4</sup> we described the prevalence of vaginal colonization of *E. coli* among 495

healthy premenopausal women and identified by univariate statistical analysis several host factors that were associated with increased vaginal carriage. In this final report we describe our findings after in-depth analysis of risk factors, using multivariate statistical methods and quantitative culture data. Demographic and host factors examined include age, menstrual cycle, sexual activity, contraceptive method, tampon use and brand, history of previous urinary tract or vaginal infection, prior use of antibiotics, and presence of genital symptoms. The influence of concurrent vaginal isolation of *Gardnerella vaginalis*, *Streptococcus agalactiae*, *Staphylococcus aureus*, and *Candida albicans* on *E. coli* colonization was also investigated. In addition, sequential and quantitative vaginal cultures were obtained from 12 unselected individuals at various intervals throughout the menstrual cycle to further delineate the quantitative relationship of vaginal *E. coli* with phases of the menstrual cycle. The finding of vaginal *E. coli* as a risk for coisolation of *Staphylococcus aureus* that is positive for the toxic shock syndrome toxin-1 has been separately reported elsewhere.<sup>5</sup>

### Materials and methods

Studies of the prevalence and colonization patterns of vaginal *E. coli* among 495 healthy women were conducted at the Student Health Services of the University

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of British Columbia between January and April, 1982. Additional vaginal cultures were obtained sequentially from 12 women in 1983 during days 5 ( $\pm 2$ ), 15 ( $\pm 2$ ), and 21 ( $\pm 2$ ) of the menstrual cycle. Before enrollment, informed consent was obtained from each participant, and a confidential questionnaire was completed with information regarding current sexual activity, methods of contraception, menstrual hygiene, previous history of genital and urinary tract infections, and recent antibiotic use. A manual pelvic examination was performed, and specimens for vaginal culture were obtained from the posterior fornix and both lateral walls of the vagina under direct visualization, with use of a sterile speculum lubricated with K-Y jelly (Johnson & Johnson, New Brunswick, New Jersey). Swabs were placed in modified Amies transport medium (Starplex Scientific, Mississauga, Ontario), refrigerated immediately, and were processed in the research laboratory within 48 hours.

Vaginal swab specimens were inoculated onto MacConkey agar as well as 5% sheep blood brain heart infusion and mannitol salt medium with use of a semi-quantitative technique, and were incubated aerobically at 37°C for 48 hours. Growth of *E. coli* on MacConkey agar was recorded as 0, 1+, 2+, 3+, and 4+, previously determined to be quantitatively equivalent to  $\leq 10^3$ ,  $10^4$ ,  $10^5$ ,  $10^6$ , and  $10^7$  colony forming units per milliliter (cfu/ml), respectively. *E. coli* was identified by the API 20E system (Analytab Products, Plainville, New York) and *S. aureus* by the API Staph-ident system (Analytab). A vaginal swab specimen obtained simultaneously from each patient was submitted separately to the diagnostic laboratory for isolation and identification of *G. vaginalis*, *S. agalactiae*, and *C. albicans* by standard procedures. For quantitative cultures sequentially obtained during the menstrual cycle, vaginal swab specimens were processed immediately in an anaerobic chamber. After elution and agitation by Vortex mixer in 1 ml of prereduced anaerobically sterilized diluent salt solution, three 100-fold serial dilutions were prepared and 0.1 ml samples were plated in prereduced anaerobically sterilized and routine medium. These media included MacConkey agar, mannitol salt agar, prereduced anaerobically sterilized brain heart infusion agar supplemented with 10% defibrinated sheep blood and vitamin K-hemin (0.01%, vol/vol), and brain heart infusion agar containing vancomycin and kanamycin (7.5 and 75  $\mu\text{g/ml}$ , respectively). Anaerobic plates were retained within the anaerobic chamber and incubated for 7 days. Aerobic plates were taken out of the chamber and incubated for 48 hours in 5% carbon dioxide or air. Colony types were enumerated and identified. Bacterial counts were expressed as  $\log_{10}$  colony forming units per milliliter of diluent. This quantitative culture technique was highly sensitive (lower limit of

detection,  $10^2$  cfu/ml) and reproducible (correlation coefficient, 0.994 by duplicate sampling in three subjects).

The production of toxic shock syndrome toxin-1 by *S. aureus* isolates was determined by analytic isoelectrofocusing of ethanol precipitates from culture filtrates,<sup>5</sup> and confirmed both by an enzyme-linked immunoabsorbent assay and by Ouchterlony gel immunodiffusion of culture filtrates as previously described.<sup>6</sup>

Statistical analysis was performed with an Amdahl computer system and the Statistical Package for the Social Sciences program. Both parametric and non-parametric methods were used where appropriate. Multivariate statistics were obtained by established techniques of multiple stepwise logistic regression. In the sequential and quantitative studies, menstrual cultures were compared with paired midcycle and premenstrual cultures from the 12 subjects by the Wilcoxon paired rank sign test.

## Results

Only two of 502 women seen consecutively for a pelvic examination at the clinic of the Student Health Services declined to participate in the study. Specimens for culture were not obtained from five women at the time the questionnaire was completed, and data on these women were excluded from our analysis. The demographic characteristics of the 495 women who completed the study have been described previously<sup>4</sup> and thus are summarized only briefly here. The mean age of the participants was 22.8 years. Twenty-eight percent of the women came to the clinic because of genital symptoms, including vaginal discharge with or without irritation, abnormal menstruation, and pelvic pain; 74% of the women attended the clinic for an annual physical examination and had no genital complaints. Seventy-eight percent of the participants used contraceptive methods, including oral contraceptive (44%), an intrauterine device (10%), condoms with or without foam (12%), and a diaphragm or cervical cap (10%). Eighty-eight percent of the participants regularly used tampons. Twenty-four percent of the women had a history of urinary tract infection, but none had experienced an episode within the preceding 2 weeks. Six percent of the participants had a vaginal infection during the preceding two weeks, and 7% had received antibiotics during the same period. Specimens for vaginal cultures were obtained during menstruation (days 1 to 7 of the menstrual cycle) from 16% of participants, after menstruation (days 8 to 21) from 54%, and before menstruation (on or after day 22) from 30%.

The prevalence rates of vaginal carriage of *E. coli* among these 495 women and the mean bacterial counts recovered are summarized in Table I. Overall, *E. coli* was isolated from 61 (21%) of the women. Enterobacteriaceae other than *E. coli* were isolated from six ad-

**Table I.** Vaginal carriage of *E. coli* among 495 healthy women

Associated factor	No. of women	Women with <i>E. coli</i>		Mean concentration (log <sub>10</sub> cfu/ml ± SEM*)
		No.	%	
Day of menstrual cycle				
1-7	79	16	20†	5.25 ± 0.25
8-14	146	22	15	5.00 ± 0.20
15-21	120	10	8	4.90 ± 0.34
≥22	150	13	9	4.92 ± 0.32
Tampon use‡				
Yes	437	51	12	5.07 ± 0.14
Tampax	211	23	11	5.56 ± 0.21
Carefree O.B.	123	16	13	5.18 ± 0.29
Playtex	71	9	13	4.66 ± 0.28§
Kotex	22	1	5	5.0
Other	37	5	13	4.40 ± 0.24§
No	57	10	17	4.80 ± 0.29
Contraception practiced				
Yes	386	53	13	5.03 ± 0.14
Oral contraceptives	220	27	12	4.89 ± 0.19
Condoms	57	5	9	5.00 ± 0.31
Intrauterine device	48	4	8	4.75 ± 0.25
Diaphragm or cervical cap	49	13	26†	5.38 ± 0.35
Other	12	4	8	5.25 ± 0.63
No	106	8	7	5.00 ± 0.38
Current sexual activity				
Yes	388	54	14	5.07 ± 0.14
No	106	7	7	4.71 ± 0.28
Intercourse during menses				
Yes	205	34	17	5.05 ± 0.18
No	179	20	11	5.20 ± 0.23
Current genital symptoms				
Present	141	25	18†	4.92 ± 0.23
Absent	350	36	10	5.11 ± 0.15
Vaginal infection during preceding 2 weeks				
Yes	31	6	19	4.66 ± 0.33
No	458	55	12	5.07 ± 0.14
Use of vaginal douche or spray				
Yes	30	3	10	5.00 ± 0.00
No	463	58	12	5.03 ± 0.14
Antibiotic use during preceding 2 weeks				
Yes	30	8	27†	5.00 ± 0.46
No	422	48	11	5.00 ± 0.14
History of urinary tract infection				
Yes	121	27	22†	4.89 ± 0.20
No	372	34	9	5.14 ± 0.17
Total (all women)	495	61	12	5.03 ± 0.13

\*SEM, standard error of the mean.

†p < 0.05, by  $\chi^2$  analysis of comparison groups within same category.

‡Multiple brands were used by 26 women.

§p &lt; 0.05, compared with Tampax users (rank sum test, two-tailed).

ditional women (13 subjects in all) and included *Klebsiella pneumoniae* (nine women), *Proteus mirabilis* (two), *Enterobacter cloacae* (one), and *Serratia rubidaea* (one). The vaginal carriage rate for *E. coli* was significantly higher among women in the menstrual or immediately postmenstrual phase of the cycle ( $p < 0.005$ ,  $\chi^2$  analysis with 3 df) and among those with prior use of antibiotics during the preceding 2 weeks ( $p < 0.05$ ), presence of genital complaints ( $p < 0.05$ ), history of previous urinary tract infection ( $p < 0.001$ ), and use of diaphragm or cervical cap for contraception ( $p < 0.005$ ). Although the rates of carriage among women who used tampons

or specific brands of tampons were not significantly different from those who used napkins exclusively, bacterial counts for *E. coli* recovered by semiquantitative culture were significantly higher among women who used Tampax exclusively, compared to those who used Playtex or other brands ( $p < 0.05$ , rank sum test).

The association of vaginal *E. coli* with coisolation of other facultative bacteria in the same specimens is summarized in Table II. *S. aureus*, *G. vaginalis*, *S. agalactiae*, and *C. albicans* were isolated from 6.8%, 17%, 10%, and 19%, respectively, of the 495 women. The rate of vaginal carriage of *E. coli* was significantly higher among

**Table II.** Coisolation of *S. aureus*, *G. vaginalis*, *S. agalactiae*, and *C. albicans* with *E. coli* from 495 healthy women

Vaginal isolate	No. of women	Women with <i>E. coli</i>		Mean concentration (log <sub>10</sub> cfu/ml ± SEM)
		No.	%	
<i>S. aureus</i>				
Toxicogenic	13	7	54*	5.43 ± 0.48†
Nontoxicogenic	20	3	15	4.00 ± 0.00
Absent	461	51	11	5.07 ± 0.14
<i>G. vaginalis</i>				
Present	84	13	14	4.61 ± 0.21
Absent	411	48	12	5.18 ± 0.15
<i>S. agalactiae</i>				
Present	52	9	17	4.66 ± 0.23
Absent	443	52	12	5.13 ± 0.14
<i>C. albicans</i>				
Present	94	14	15	4.61 ± 0.18
Absent	401	47	12	5.18 ± 0.15

\* $p < 0.05$  ( $\chi^2$  with Yates' correction), compared to prevalence rate in women with nontoxicogenic *S. aureus*, and  $p < 0.0005$ , compared to prevalence rate in women without *S. aureus*.

† $p < 0.05$  (rank sum test, one-tailed) compared to women with nontoxicogenic *S. aureus*.

**Table III.** Significant correlations with vaginal carriage of *E. coli*

Associated factors	Women with <i>E. coli</i>		<i>p</i> value	
	No.	%	Univariate*	Multivariate†
Day of menstrual cycle				
1-7	16	20	<0.005	<0.05
8-14	22	15		
15-21	10	8		
≥22	13	9		
Method of contraception				
Diaphragm or cervical cap	13	26	<0.005	<0.05
Other	40	12		
None	8	7		
Current genital symptoms				
Yes	25	18	<0.05	NS
No	36	10		
Prior use of antibiotics				
Yes	8	27	<0.05	<0.05
No	48	11		
History of urinary tract infection				
Yes	27	22	<0.001	<0.001
No	34	9		
Vaginal <i>S. aureus</i> coisolated				
Toxicogenic	7	54	<0.005 ↓	<0.05 ↓
Nontoxicogenic	3	15		
None	51	11		

NS = Not significant.

\* $\chi^2$  with Yates' correction.

†Multiple stepwise logistic regression analysis.

women who carried *S. aureus* that was positive for toxic shock syndrome toxin-1, compared to women who carried either nontoxicogenic strains of *S. aureus* ( $p < 0.05$ ) or no *S. aureus* at all ( $p < 0.0005$ ). No significant differences in rates of isolation of *E. coli* were noted among women with or without coexisting *G. vaginalis*, *S. agalactiae*, or *C. albicans*.

Factors that were significantly associated with vaginal carriage of *E. coli* by univariate analysis were further examined by multiple stepwise logistic regression to eliminate the influence of confounding variables. Phase

of menstrual cycle, use of diaphragm or cervical cap for contraception, prior use of antibiotics, history of urinary tract infection, and coisolation of *S. aureus* positive for toxic shock syndrome toxin-1 remained as significant factors by multivariate analysis ( $p < 0.05$ ) (Table III). The relative odds for vaginal carriage of *E. coli* associated with these determinants are summarized in Table IV.

The influence of the menstrual cycle on vaginal carriage of *E. coli* as well as other facultative and anaerobic bacteria was separately studied by quantitative and se-



**Table IV.** Odds ratios for vaginal carriage of *E. coli*

Factors	Odds ratio	90% confidence interval	p value*
History of urinary tract infection	2.47	1.44-4.24	<0.025
Diaphragm or cervical cap versus no contraception	6.09	2.29-16.19	<0.025
Vaginal <i>S. aureus</i> present			
Toxicogenic versus none	8.17	2.77-23.95	<0.001
Toxicogenic versus nontoxicogenic	11.02	1.97-61.72	<0.025
Prior use of antibiotics	2.77	1.06-7.20	<0.025
Day of menstrual cycle			
1-7 versus $\geq 22$	4.45	1.13-17.46	<0.05

\*One-tailed.

**Table V.** Sequential and quantitative vaginal cultures obtained from 12 healthy women at different days of the menstrual cycle

Vaginal isolates	Day 5 $\pm$ 2		Day 15 $\pm$ 2		Day 21 $\pm$ 2	
	No. of women	Mean concentration ( $\log_{10}$ cfu/ml $\pm$ SEM)	No. of women	Mean concentration ( $\log_{10}$ cfu/ml $\pm$ SEM)	No. of women	Mean concentration ( $\log_{10}$ cfu/ml $\pm$ SEM)
Anaerobes	12	6.25 $\pm$ 0.50	12	7.16 $\pm$ 0.24*‡	12	7.0 $\pm$ 0.32
Gram-positive cocci	4	4.12 $\pm$ 0.59	3	6.66 $\pm$ 0.67	4	5.66 $\pm$ 1.19
Gram-positive bacilli	4	4.50 $\pm$ 1.19	5	5.80 $\pm$ 0.79	8	5.37 $\pm$ 0.70
Gram-negative cocci	7	4.92 $\pm$ 0.79	3	7.33 $\pm$ 0.61	6	6.33 $\pm$ 0.80
Gram-negative bacilli	10	5.85 $\pm$ 0.72	10	7.20 $\pm$ 0.24†§	6	7.16 $\pm$ 0.16
Aerobes	12	6.12 $\pm$ 0.36	12	5.66 $\pm$ 0.61	12	6.25 $\pm$ 0.66
Gram-positive cocci	12	4.91 $\pm$ 0.41†‡	11	4.81 $\pm$ 0.50	11	3.45 $\pm$ 0.62
Gram-positive bacilli	7	4.85 $\pm$ 0.72	9	4.11 $\pm$ 0.73	6	6.83 $\pm$ 0.40
Gram-negative cocci	2	3.75 $\pm$ 0.24	2	3.0 $\pm$ 0.00	1	7.0
Gram-negative bacilli	9	6.72 $\pm$ 0.42	9	5.00 $\pm$ 0.72	9	5.44 $\pm$ 0.89
<i>E. coli</i>	6	5.33 $\pm$ 0.42†‡	5	4.40 $\pm$ 1.07†‡	4	2.57 $\pm$ 1.16

\*Colony counts significantly higher than menstrual (day 5  $\pm$  2) cultures, by Wilcoxon paired rank sign test (one-tailed).†Colony counts significantly higher than premenstrual (day 21  $\pm$  2) cultures, by Wilcoxon paired rank sign test (one-tailed).

‡p &lt; 0.05.

§p &lt; 0.025.

quential cultures in 12 healthy women (Table V). The mean age of these women was 23 years; all used tampons regularly; and all except one practiced contraception (oral contraceptives, 7; intrauterine device, 3; diaphragm, 1). Colony counts of *E. coli* were higher in either menstrual (day 5  $\pm$  2) or midcycle (day 15  $\pm$  2) samples compared to paired premenstrual (day 21  $\pm$  2) specimens (p < 0.05, Wilcoxon paired rank sign test, one-tailed). Similarly, colony counts of aerobic gram-positive cocci were higher in menstrual sample compared to paired premenstrual specimens (p < 0.05), whereas those of anaerobic gram-negative bacilli were higher in midcycle samples compared to paired premenstrual specimens (p < 0.025). In addition, the total anaerobic counts were higher in midcycle samples than in paired menstrual specimens (p < 0.05).

#### Comment

We have further defined the epidemiologic characteristics of vaginal carriage of *E. coli* in healthy premenopausal women. Our finding of an overall preva-

lence rate of 12% is similar to that reported by others in comparable study populations (6% to 26%).<sup>1,3</sup> The relatively low carriage rate indicates that this organism should not be considered as part of the normal indigenous vaginal flora in healthy premenopausal women. In contrast, vaginal carriage rates of *E. coli* are conspicuously higher during the prepubertal (15% to 90%) and postpartum (30% to 41%) periods<sup>7</sup> and following vaginal hysterectomy (72% to 75%).<sup>8</sup> It is interesting that several studies indicate that vaginal isolation rates of *E. coli* are not increased among women with vaginitis or abnormal vaginal discharge (8% to 16%) compared to asymptomatic women.<sup>3,9</sup> Vaginal carriage of *E. coli* in menopausal women, with or without estrogen replacement, also does not appear markedly increased (21%-32%).<sup>10</sup>

Several factors were identified to be significantly associated with vaginal colonization by *E. coli* when confounding variables were controlled by multivariate analysis. These include phase of the menstrual cycle, prior use of antibiotics, use of diaphragm or cervical

cap for contraception, history of urinary tract infection, and coisolation of *S. aureus* that is positive for toxic shock syndrome toxin-1. The significant association of vaginal carriage of *E. coli* with toxicogenic *S. aureus* (but not nontoxicogenic *S. aureus*) is unexpected and has been reported separately.<sup>5</sup> This did not appear to be a chance association, since a similar relationship was noted for Enterobacteriaceae other than *E. coli* and toxicogenic *S. aureus* but not for *G. vaginalis*, *S. agalactiae*, or *C. albicans*.<sup>5</sup> The biologic basis of this association remains unexplained.

Our observation that both the carriage rates and bacterial counts of vaginal *E. coli* were significantly higher in menstrual than in premenstrual cultures further indicates the hormonal influence on vaginal colonization by *E. coli*. Larsen and Galask<sup>11</sup> and Botta et al.<sup>12</sup> have also noted considerably higher recovery rates for vaginal *E. coli* in menstrual (28% to 36%) than in premenstrual cultures (0% to 13%). In vitro adherence studies also indicate that the receptiveness of vaginal and uroepithelial cells for both *E. coli*<sup>13</sup> and *S. agalactiae*<sup>14</sup> may fluctuate during the menstrual cycle. It is of interest that this fluctuation in vaginal colonization by Enterobacteriaceae observed in healthy women during the menstrual cycle appears to be aborted in women prone to recurrent urinary tract infection. Botta et al.<sup>12</sup> studied quantitative vaginal cultures from 15 healthy women and nine women with recurrent urinary tract infection sequentially during the menstrual cycle. None used oral contraceptives or intrauterine devices and all were otherwise symptom-free. At ovulation both the carriage rates and colony counts for Enterobacteriaceae were higher in the urinary tract infection group than in health control subjects (80% compared to 35%). During the premenstrual period, however, the high Enterobacteriaceae counts rapidly declined in control women but persisted in the urinary tract infection group. These data, and our finding of a 2.5-fold higher relative odds for vaginal *E. coli* in women with a history of previous urinary tract infection, are consistent with the observation of Stamey and Sexton<sup>15</sup> that women with introital *E. coli* are more prone to develop recurrent urinary tract infection.

Of considerable interest is our finding that vaginal carriage of *E. coli* is significantly associated with use of a diaphragm or cervical cap for contraception. This association, previously reported by us in a preliminary communication,<sup>4</sup> is reaffirmed in the present study and conclusively demonstrated by multivariate statistical analysis as a significant and independent variable. This is important, since we found that women who used a diaphragm or cervical cap were also more likely to have a history of urinary tract infection (37% of 49 users versus 23% of 444 nonusers,  $p < 0.05$ ). These observations may explain the findings of the long-term fol-

low-up study of the Oxford Family Planning Association that women using a diaphragm for contraception had a higher incidence of urinary tract infection compared to women using other contraceptive methods.<sup>16</sup> The diaphragm and cervical cap are generally used in conjunction with spermicidal preparations with detergent properties that may alter epithelial cell surfaces and favor adherence and colonization by *E. coli*.

Our quantitative studies of the vaginal microflora in 12 healthy women indicate that both aerobic and anaerobic bacterial counts remained high throughout the menstrual cycle. As occurred in the studies of Bartlett et al.,<sup>2</sup> a remarkable consistency was observed in the recovery of major categories of organisms from a particular subject, despite great variations in the isolation of specific bacteria in different samples of a given individual. Our findings differed from Bartlett et al.<sup>2</sup> in that although bacterial counts for both *E. coli* and aerobic gram-positive cocci significantly decreased in premenstrual samples compared to menstrual or midcycle specimens, the total aerobic counts were not significantly decreased in premenstrual specimens. Counts for anaerobic gram-negative bacilli were significantly higher in midcycle samples compared to premenstrual cultures in our study, whereas the total anaerobic counts were also highest during midcycle.

Since the indigenous microflora appear to be a major host defense mechanism against invading pathogens, further efforts to characterize the complete vaginal flora and identify the important determinants of "colonization resistance" in health and disease are clearly warranted.

#### Addendum

Since submission of this manuscript, Fihn et al. have also reported a significant association between diaphragm use and urinary tract infection both in case-control and retrospective cohort studies (JAMA 1985; 254:240).

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## Maternal-fetal ABO/Rh antigenic relationships and human fetal development

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Six hundred ninety-nine primigravid mothers and their neonates were grouped into three distinct classes on the basis of maternal-fetal antigenic relationships for the ABO and Rh blood groups. Maternal-fetal units were classified as maternal dominant if the mother possessed more antigens than the neonate, equivalent if both possessed the same number of antigens, and fetal dominant if the neonate possessed more antigens than the mother. After confounding variables were controlled, a significant increase in adjusted mean birth weight was observed from maternal-fetal equivalence to fetal dominance and there was a significant increase in adjusted crown-heel length from maternal dominance to fetal dominance. No trends were observed for adjusted head circumference. However, when the sample was stratified on the basis of sex of the neonate, a highly significant increase in adjusted mean head circumference was observed for female infants from maternal dominance through fetal dominance. These observations suggest maternal-fetal ABO/Rh relationships are associated with differential fetal growth trends. (*AM J OBSTET GYNECOL* 1986;154:126-9.)

**Key words:** Maternal-fetal antigens, ABO blood group, Rh factor

Studies among experimental animals have shown that the greater the degree of antigenic dissimilarity between the blastocyst and the mother, the greater the likelihood of implantation and survival and the faster the rate of placental and fetal development.<sup>1,3</sup> There is

some evidence that this results from the masking or removal of trophoblastic alloantigens by maternal IgG "blocking" antibodies, which are able to cross the placenta.<sup>4,5</sup> Another possible mechanism of immunosuppression may be the generation of maternal suppressor T cells, which results in the inhibition of an endometrial lymphocytotoxic response.<sup>6</sup> Hormonal and other agents also appear to play a role in inhibition of a maternal lymphocytotoxic response to the fetoplacental unit,<sup>4,6</sup> and later in development the fetus may produce immunosuppressive factors as well.<sup>7</sup>

It is not clear how this same process would promote fetal and placental growth enhancement. Some possibilities are (1) that the maternal immune system places

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constraints on fetal growth and immunosuppression removes some of these constraints, (2) that immune agents (immunoglobulins?) act directly as growth promoters, or (3) that growth-promoting factors are produced as a byproduct of immunosuppressive events.<sup>4</sup>

It is likely that maternal-fetal antigenic differences also play an influential role in conception, implantation, and survival of the human embryo and fetus. However, results of investigations concerning the association between maternal-fetal antigenic relationships and human fetoplacental development are conflicting.<sup>6</sup> In an examination of maternal ABO blood group status and placental weights, Jones<sup>8</sup> found that mothers with type O blood, who would be expected to have the highest frequency of antigenically dissimilar fetuses, in fact produced smaller placentas on the average than mothers with Types A, B, and AB. Seppala and Tolonen<sup>9</sup> failed to find any association of maternal-fetal ABO dissimilarity with fetal or placental development. On the other hand, using reactivity in bidirectional mixed maternofetal lymphocyte cultures as a measure of antigenic dissimilarity, Jenkins and Good<sup>10</sup> observed a significant association between the degree of maternal-fetal antigenic dissimilarity and an increase in placental weight in humans.

Because we had access to pregnancy outcome data (including maternal and neonatal ABO/Rh blood group types) in a large sample of primigravid women, we decided to investigate whether an association existed between maternal-fetal ABO/Rh antigenic relationships and fetal development as assessed by size of the neonate in respect to gestational age. Although such an association has been proposed,<sup>11</sup> to date there are not sufficient data to support this hypothesis.<sup>6</sup>

## Methods

Most previous studies have compared fetal outcome in those situations in which mother and fetus share antigens in common with those situations in which the fetus possesses paternally derived antigens that are foreign to the mother (alloantigens). To our knowledge, no studies have investigated those situations in which the mother possesses antigens not inherited by (and thus foreign to) the fetus. As a result, we decided to quantify the magnitude and direction of maternofetal antigenic differences in our investigation of the ABO and Rh loci. By magnitude, we mean the net difference between the mother and fetus in the amount of immunoreactive ABO/Rh alloantigens they possess. By direction, we mean whether it is the mother or the fetus that has the relatively greater number of immunoreactive antigens. We introduce the concept of the antigenic vector and its related terms, maternal dominance, maternal-fetal equivalence, and fetal dominance, to refer to the direction and magnitude of maternal-fetal relationship. We use the term dominance to refer to the

situation in which the mother (maternal dominance) or the fetus (fetal dominance) possesses the relatively greater number of alloantigens. When the mother and fetus possess the same number of alloantigens, this is referred to as maternal-fetal equivalence.

With the fetus used as the reference point, we developed a scale of antigenic vector scores ranging from maternal to fetal dominance. Computation of the scores was designed to quantify the net difference between fetus and mother in the number (but not necessarily the type) of immunoreactive ABO/Rh antigens. The O (H antigen) and Rh negative (d antigen) phenotypes were considered to be antigenically silent, since these antigens are not strongly immunoreactive. To illustrate, if the fetus possesses two immunoreactive antigens (for instance, A and Rh +) and the mother one (for instance, B and Rh -), the maternal-fetal antigenic vector score equals +1 (fetal dominance); if the mother has two immunoreactive antigens (for instance, B and Rh +) and the fetus none (for instance, O and Rh -), the maternal-fetal antigenic vector score equals -2 (maternal dominance). Maternal-fetal equivalence occurs when the mother and fetus possess the same number of immunoreactive antigens (none, one, or two) and, hence, the antigenic vector score equals 0. Antigenic difference scores were calculated for each maternal-fetal unit for the combined ABO and Rh antigenic phenotypes. Because of small sample sizes for the antigenic vector score = -2 ( $n = 3$ ) and antigenic vector score = +2 ( $n = 14$ ) categories, maternal-fetal units with these values were pooled with the antigenic vector score = -1 and antigenic vector score = +1 categories, respectively.

The sample used in this study consisted of 699 black and white primigravid women, 12 to 31 years of age, who gave birth at the University of South Alabama Medical Center between 1974 and 1980. Primigravid women were chosen in order to minimize the possible cumulative effects of repeat pregnancies on the intensity of immunologic interactions between mother and fetus. All subjects were women from a lower socioeconomic background who had been enrolled in a Maternal and Infant Care prenatal clinic at the Mobile County Health Department. These women were also enrolled in a Women, Infants and Children dietary supplementation program.

Information on blood type of mother and fetus was recorded directly from the obstetric and neonatal records of the University of South Alabama Medical Center. In order to estimate fetal growth and development, we recorded the gestational age (based on last menstrual period), birth weight, crown-heel length, and head circumference dimensions of the neonates in our study. Data on potential confounding variables associated with fetal development, such as gestational weight gain, number of prenatal visits, and levels of cigarette

**Table I.** Pairwise comparisons of birth weight with the sexes pooled between antigenic vector score\* groups by analysis of covariance

Antigenic vector score	n	Adjusted mean† (gm)	Trend, F	p
-1	64	3120	0.98	NS
0	411	3100		
+1	224	3180		

\*See Methods for explanation.

†Adjusted for gestational age, sex of neonate, smoking level, number of prenatal visits, and gestational weight gain.

**Table II.** Pairwise comparisons of crown-heel length with the sexes pooled between antigenic vector score\* groups by analysis of covariance

Antigenic vector score	n	Adjusted mean† (cm)	Trend, F	p
-1	63	50.6	4.71	<0.0305
0	397	51.1		
+1	217	51.4		

\*See Methods for explanation.

†Adjusted for gestational age, sex of neonate, smoking level, number of prenatal visits, and gestational weight gain.

smoking (no cigarettes, less than a half pack per day, or a half pack or more per day) were also recorded in order to control for these variables in the statistical analysis.

Trends in fetal development were examined by analysis of covariance,<sup>12</sup> wherein mean values of the maternal dominant (antigenic vector score = -1), maternal-fetal equivalent (antigenic vector score = 0), and fetal dominant (antigenic vector score = +1) groups were compared after gestational age, sex of the neonate, and the confounding variables were adjusted. This was done for all neonates (sexes pooled) and for neonates when separated (stratified) into two different subsamples by sex of the neonate (sex not being a covariate in these instances). From this point we shall frequently use the term increasing fetal dominance when referring to trends across the antigenic vector score scale from maternal dominance (-1) to fetal dominance (+1).

## Results

When adjusted mean birth weights were compared between maternal dominant and fetal dominant groups, there was a tendency for birth weights to increase from maternal dominance and maternal-fetal equivalence to fetal dominance (see Table I). The difference between antigenic vector score = 0 and anti-

**Table III.** Pairwise comparisons of head circumference between antigenic vector score\* groups by analysis of covariance

Antigenic vector score	n	Adjusted mean† (gm)	Trend, F	p
<i>Sexes pooled</i>				
-1	63	32.9	0.14	NS
0	395	32.8		
+1	217	33.0		
<i>Female neonates</i>				
-1	30	32.1	7.08	<0.0081
0	185	32.5		
+1	103	33.0		

\*See Methods for explanation.

†Sexes pooled: Adjusted for gestational age, sex of neonate, smoking level, number of prenatal visits, and gestational weight gain. Female neonates: Adjusted for gestational age, smoking level, number of prenatal visits, and gestational weight gain.

genic vector score = +1 was statistically significant ( $p < 0.0223$ ). When the sample was stratified on the basis of sex, the birth weight trend was significant for female infants from antigenic vector score = 0 to antigenic vector score = +1 ( $p < 0.0145$ , results not presented). A significant upward trend from antigenic vector score = -1 to antigenic vector score = +1 was observed for adjusted crown-heel length means ( $p < 0.0305$ ) (see Table II). When the sample was stratified by sex of the neonate, this trend was nearly significant for female neonates ( $p < 0.0745$ ) from antigenic vector score = -1 to antigenic vector score = +1 (results not presented). No significant trend was observed for adjusted head circumference values for the total sample. However, when the neonates were stratified by sex, a highly significant increase was observed for female neonates with increasing fetal dominance from antigenic vector score = -1 to antigenic vector score = +1 ( $p < 0.0081$ ) and from antigenic vector score = 0 to antigenic vector score = +1 ( $p < 0.0075$ ) (see Table III).

## Comment

The results of the analyses of covariance suggest that there is (1) a significant increase in birth weight and crown-heel length with increasing fetal dominance and (2) a significant increase in head circumference for female infants with increasing fetal dominance. The overall picture is one that supports the hypothesis that maternal-fetal ABO/Rh antigenic relationships play an influential role in human fetal development. This hypothesis is further supported by the findings from like

studies among experimental animals for the major histocompatibility antigens.<sup>14</sup> What mechanism might favor a comparatively faster growth rate when the fetus is dominant over the mother is not known. The fact that there is an immunologic basis of this phenomenon has been inferred by observations that maternal IgG blocking antibodies (besides their apparent immunosuppressive action) facilitate the growth of antigenically foreign cells<sup>13</sup> and promote tumor growth in vitro.<sup>14</sup>

Results of stratification of the sample by sex of the neonate suggest that male fetuses are affected by ABO/Rh antigenic dissimilarity but perhaps not as much as female fetuses. To our knowledge, sex differentials in growth associated with maternal-fetal incompatibility have not previously been reported or, for that matter, even examined. Findings of such sex differences are consistent with the antigenic vector hypothesis, since possession of the immunogenic H-Y antigens makes male fetuses more antigenically dissimilar to their mothers, on the average, than female fetuses.<sup>15, 16</sup> Therefore, it is possible that the presence of the H-Y antigens alters immunologic interactions because of ABO/Rh maternal-fetal differences for male neonates. This could obscure the association between these antigenic differences and fetal development and explain the observations of weaker statistical associations for male neonates.

Finally, another interesting consequence of the findings in our study is the possibility that there exists a continuum of effect of maternal-fetal antigenic dissimilarity on fetal development from maternal to fetal dominance, with intrauterine growth retardation being more likely when the mother is "dominant" over the fetus. In other words, the concept of antigenic vector (a statistical artifice represented by the antigenic vector scores) may reflect an underlying biologic process that plays an influential role in mammalian embryogenesis.

Further studies are warranted to establish whether this latter finding is a general phenomenon of mammalian fetal development.

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# Epithelial regulation of prolactin effect on amnionic permeability

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The permeability of human amnion to tritiated water is reduced in the presence of both human and ovine prolactin. The cellular composition of amnion is such that the action of prolactin on this membrane probably occurs by way of the epithelium lining the fetal surface. The present study sought to confirm an epithelial site of action of prolactin on the permeability of amnionic membrane to tritiated water. In addition, radioautography and competition experiments were conducted to determine a possible receptor-mediated mechanism for prolactin action. Membrane permeability to tritiated water was found to be equivalent for both intact membranes and membranes enzymatically stripped of the lining epithelial cells. However, when ovine prolactin was presented to the fetal surface of amnion, only intact membrane displayed decreased permeability to tritiated water. Although localization of iodine 125-labeled prolactin to the light cell population of amniotic epithelium was observed, positive evidence of a receptor-mediated mechanism could not be established. The results indicate that the permeability of human amnion to water is influenced principally by cells of the epithelium in response to prolactin. (AM J OBSTET GYNECOL 1986;154:130-4.)

**Key words:** Amnion, epithelium, prolactin

During human pregnancy decidualized endometrium produces prolactin that accesses the amniotic fluid following transport across the fetal membranes.<sup>1,2</sup> A number of studies have demonstrated that human and ovine prolactin presented to the fetal surface of human amnion significantly reduce the permeability of this membrane to tritiated water in the fetal to maternal direction.<sup>3-5</sup> Although both lactogenic and human prolactin-specific receptors have been reported in chorion laeve and decidua, attempts to identify lactogen receptors in human amnion have been unsuccessful.<sup>6</sup> However, of the two identifiable cell types of amniotic epithelium,<sup>7</sup> only light cells selectively localize human prolactin.<sup>8</sup> Since the subepithelial component of amnion consists principally of collagen interspersed with fibroblasts and accounts for  $\geq 90\%$  of the tissue mass of amnion, we hypothesized that the site of action of prolactin on amnion resides at the level of the epithelial surface lining the fetal side.

The following study attempted to demonstrate the biologic action of prolactin on reflected amnionic membrane to be mediated by the surface epithelium. In addition, radioautography was used to assess possible evidence of prolactin receptors in amnionic epithelial cells.

## Material and methods

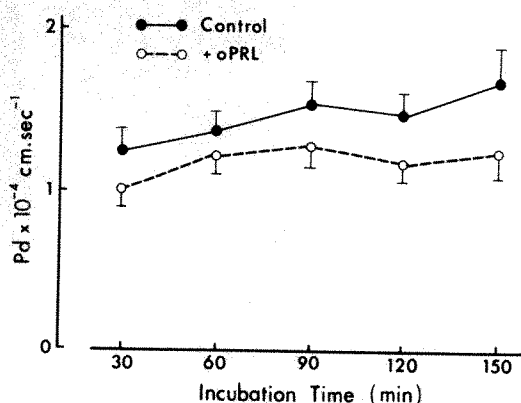
Reflected fetal membrane was cut free from fresh placentas delivered at the time of uncomplicated primary or repeat elective cesarean sections performed between 38 to 39 weeks of gestation. Membranes were rinsed free of blood in phosphate-buffered saline solution maintained at pH 7.4. Subsequently, amnion was carefully separated from choriodecidua and secured in a two-sided perfusion apparatus as previously described.<sup>2</sup> The membranes were allowed to preincubate at 20° C for 1 hour followed by a change of media. Incubation media consisted of a synthetic amniotic fluid as described by Schwartz et al.<sup>9</sup> Tritiated water (40,000 dpm) or tritiated water + 10  $\mu\text{g/ml}$  of highly purified ( $<0.5\%$  vasopressin on a molar basis) ovine prolactin (National Institutes of Arthritis, Diabetes, and Digestive and Kidney Diseases oPRL-15) was added to the fetal chamber. During incubation at 37° C the media in both chambers was continuously aerated and mixed with a humidified combination of 95% oxygen and 5% carbon dioxide. Aliquots (100  $\mu\text{l}$ ) were removed from each of fetal and maternal chambers at 0 time and every 30 minutes thereafter up to 150 minutes of incubation. Tritiated water was quantitated by liquid scintillation (in disintegrations per minute) for 2 minutes, and permeability constants were calculated from serial samples from each side according to a modified Fick equation.<sup>3</sup> Statistical comparison between permeability constants of tritiated water from amnion incubated in the absence or presence of ovine prolactin were evaluated and analyzed by a two-way analysis of variance.

**Permeability of deepithelialized amnion.** Additional experiments were performed with use of amniotic

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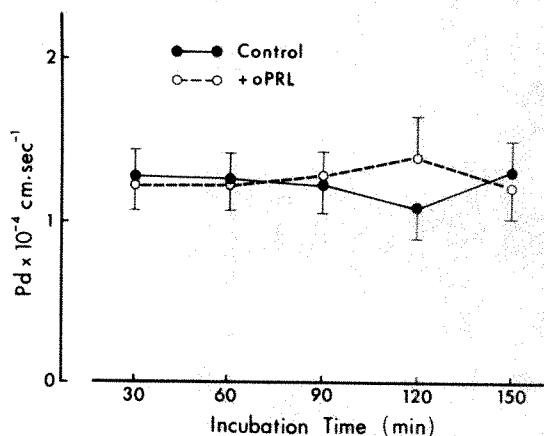
**Fig. 1.** Permeability of normal amnion to tritiated water in the absence (●—●) or presence (○---○) of 10 µg/ml of ovine prolactin. Each line represents mean permeability  $\pm$  SEM obtained from 30 membranes.

membrane enzymatically stripped of the surface epithelium by trypsinization at room temperature according to the method of Schmidt.<sup>10</sup> Synthetic amniotic fluid containing trypsin was maintained at pH 7.5 and contained 20,000 IU of penicillin and 20,000 µg/ml of streptomycin. Subsequently membranes were incubated according to the methods described above.

**Radioautography.** To determine whether localization of prolactin in amniotic epithelium is receptor-mediated, purified human and ovine prolactin were iodinated by the lactoperoxidase method.<sup>11</sup> Specific activity of the labeled hormone was determined following gel filtration through Sephadex G-50 and selection of the most active eluted fractions. Amnion was mounted in perfusion chambers and radiolabeled human or ovine prolactin was added to the fetal side of membranes. Unlabeled ovine prolactin in concentrations of 1- to 10,000-fold excess was added to the incubation media containing radiolabeled hormone. Subsequent to an incubation of 4 hours, membranes were rinsed for 10 seconds in phosphate-buffered saline solution and fixed in 10% neutral buffered formalin for 24 hours and prepared for radioautography.<sup>12</sup> Following development, radioautography sections were stained with 1% toluidine blue.<sup>7</sup> Background controls included tissues exposed to equivalent disintegrations per minute of iodine 125 sodium and treated in a similar manner. Attempts to dissociate a possible high-affinity prolactin-receptor complex included incubation of membranes pretreated with magnesium chloride in concentrations of 0, 1, and 3 mol/L as described elsewhere.<sup>13</sup> Following pretreatment, amniotic membranes were incubated according to the schedule outlined above.

## Results

Calculated permeability constants of normal amnion exposed to tritiated water either in the presence or absence of ovine prolactin are presented in Fig. 1. Two-



**Fig. 2.** Permeability of deepithelialized amnion to tritiated water in the presence (○---○) and absence (●—●) of 10 µg/ml of ovine prolactin ( $n = 13$  and  $12$ , respectively).

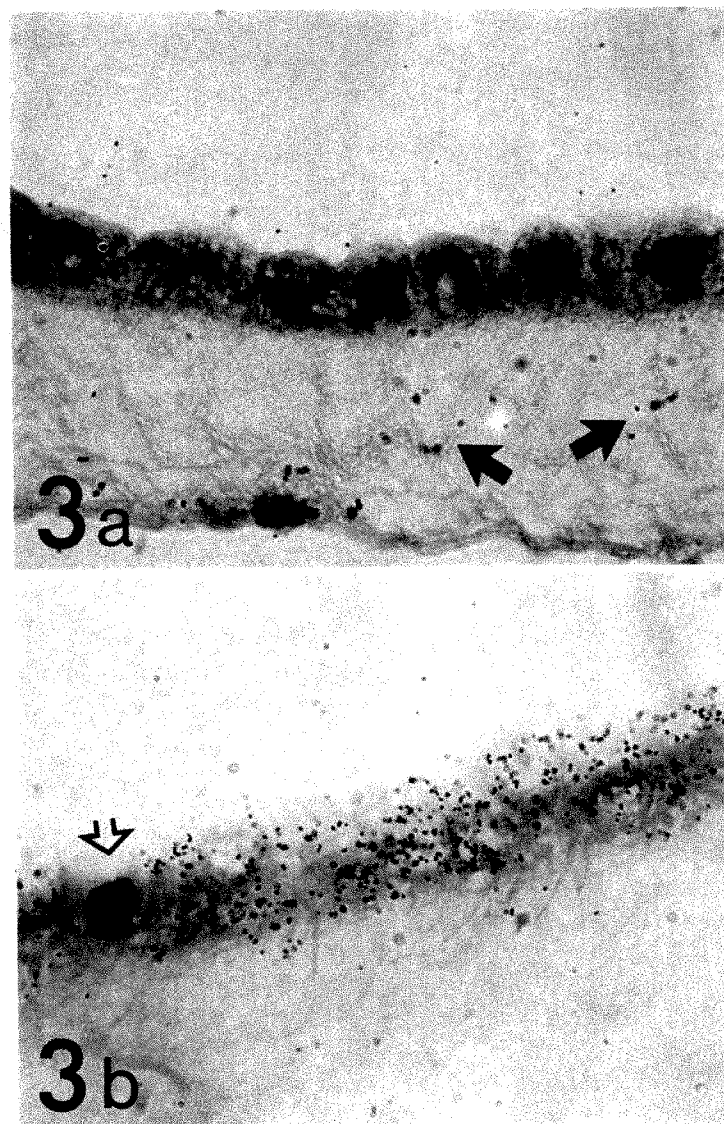
**Table I.** Competitive binding of <sup>125</sup>I-labeled ovine prolactin (oPRL) (1 ng/ml) to amniotic membranes after 3 mol/L magnesium chloride dissociation

Hormone	Radioactive uptake of <sup>125</sup> I (dpm) $\pm$ SEM
<sup>125</sup> I-oPRL (1 ng/ml) (control)	16,014 $\pm$ 1324
<sup>125</sup> I-oPRL + 1 $\times$ oPRL	15,759 $\pm$ 2038
<sup>125</sup> I-oPRL + 100 $\times$ oPRL	18,559 $\pm$ 1860
<sup>125</sup> I-oPRL + 1000 $\times$ oPRL	17,973 $\pm$ 2285
<sup>125</sup> I-oPRL + 10,000 $\times$ oPRL	16,938 $\pm$ 2487

Each value represents the mean  $\pm$  SEM of five membranes except the control value, which represents the mean  $\pm$  SEM of nine membranes (1 ng/ml of <sup>125</sup>I-oPRL  $\approx$   $2.2 \times 10^5$  dpm).

way analysis of variance identified a significant reduction in amniotic permeability to tritiated water in the presence of ovine prolactin ( $F_{\text{treatment}} = 184.5$  [1,2];  $p < 0.01$ ). A comparison between deepithelialized amnion incubated in the absence versus the presence of ovine prolactin indicated no significant difference ( $F_{\text{treatment}} = 0.05$  [1,3]; NS) in membrane permeability to tritiated water (Fig. 2). No significant difference in permeability was detectable between intact and deepithelialized amnion incubated in the absence of ovine prolactin ( $F_{\text{treatment}} = 2.87$  [1,8]). Examination of stained whole mounts of deepithelialized membranes revealed  $\geq 90\%$  dissociation of the epithelial cells from the membrane surface.

Amnion exposed to either <sup>125</sup>I-labeled human prolactin or <sup>125</sup>I-labeled ovine prolactin on the fetal surface revealed localization of hormone by the epithelial light cells. Membranes exposed to labeled ovine prolactin plus competing levels of unlabeled hormone (up to a 10,000-fold excess) failed to display decreased uptake of labeled prolactin by light cells (Fig. 3). Amniotic membranes preincubated with excess unlabeled ovine



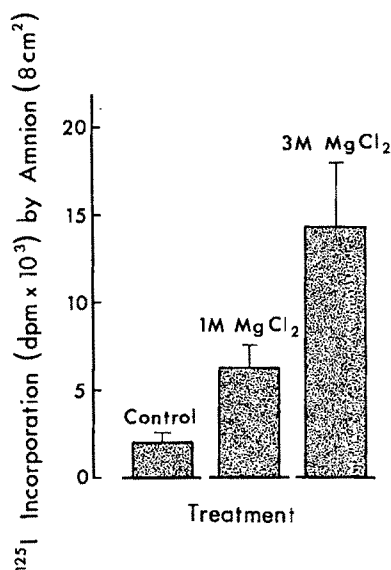
**Fig. 3.** Radioautography studies of control amnion (a) exposed to iodine 125 sodium for 4 hours showing background grains only (arrows). Amnion exposed to  $^{125}\text{I}$ -labeled ovine prolactin on the fetal surface (b) demonstrating specific localization of the hormone by light cells. Addition of unlabeled ovine prolactin resulted in no reduction in grain localization. Dark cells fail to show grain localization (arrow). (1% Toluidine blue.  $\times 400$ .)

prolactin ( $10\text{ }\mu\text{g/ml}$ ) for 1 hour and subsequently exposed to  $1\text{ ng/ml}$  of  $^{125}\text{I}$ -labeled human prolactin (approximately  $2.2 \times 10^5\text{ cpm}$ ) for 4 hours showed a slight decrease in uptake of radioactivity, but the difference was not significant. Membranes pretreated with  $1\text{ mol/L}$  or  $3\text{ mol/L}$  of magnesium chloride and subsequently exposed to  $^{125}\text{I}$ -labeled ovine prolactin ( $1\text{ ng/ml}$ ) on the fetal surface for 4 hours displayed an increased uptake of radiolabeled hormone (Fig. 4), the difference between control and  $3\text{ mol/L}$  of magnesium chloride being significantly different ( $p < 0.05$ ).

However, although magnesium chloride appeared to dissociate endogenous prolactin from amnion, allowing

increased uptake of radiolabeled hormone, competition experiments of membranes so treated showed no significant difference in  $^{125}\text{I}$ -labeled ovine prolactin uptake even with the addition of a 10,000-fold excess of unlabeled ovine prolactin (Table I).

Regardless of the procedure used, examination of radioautography results failed to identify any alteration in the number of grains present in the light cells whether amnion was incubated in the presence of  $^{125}\text{I}$ -labeled ovine prolactin with or without excess unlabeled ovine prolactin. Equivalent results were observed where  $^{125}\text{I}$ -labeled human prolactin was used with excess ovine prolactin.



**Fig. 4.** Effect of 1 mol/L and 3 mol/L of magnesium chloride treatment on the uptake of <sup>125</sup>I-labeled ovine prolactin by intact amniotic membrane. Each membrane was exposed to  $1.3 \times 10^5$  dpm of <sup>125</sup>I-labeled ovine prolactin on the fetal surface for 4 hours at 37°C. Each bar represents the mean  $\pm$  SEM for 10 amniotic membranes. F value = 6.78,  $p < 0.05$  between control and 3 mol/L of magnesium chloride.

### Comment

A significant reduction in membrane permeability to tritiated water was found for amnion exposed to prolactin on the fetal surface. Furthermore, the site of action of prolactin appears to be at the level of the amniotic epithelium. Previous studies have identified alterations in amniotic permeability to water following prolactin exposure.<sup>3,5</sup> The finding of human prolactin and ovine prolactin localization by epithelial light cells supports the view that the epithelium is regulatory on the permeability of amnion to water. However, results from both this study and other studies<sup>6</sup> suggest that prolactin action on amnion is nonreceptor mediated. It is possible that the mechanism of prolactin action involves its internalization by epithelial cells or that prolactin is irreversibly bound to a receptor.<sup>14</sup>

Permeability constants of amnion stripped of the epithelial surface were equivalent to intact membrane. The results of those experiments were unexpected, since removal of a cellular barrier suggested to us that membrane permeability would increase. However, in recent studies (unpublished observations) we found that rendering intact amnion metabolically inert by addition of sodium azide to both fetal and maternal sides failed to change the permeability of amnion relative to live membrane incubated in osmotically equivalent medium. Thus the porosity of amniotic epithelium in the absence of a hormonal stimulus may be so great as to

be inconsequential to the rate of flow of tritiated water, although bulk flow may increase.

The exchange of water across reflected fetal membrane may be influenced more by chorion laeve than by amnion alone. We recently found that the permeability of composite amniochorion to tritiated water differs from amnion when prolactin is added.<sup>5</sup> The effect of prolactin on amniochorion is seen only when the polypeptide is added to the maternal or decidual side. Thus, although amnion is responsive to prolactin, the role of amnion in determining the rate of water flow across composite membrane may be of little significance. However, amnion could affect net diffusion of water across composite fetal membrane without an alteration in the rate of molecular water flow,<sup>15</sup> since permeability constants depend on pore size rather than number of pores. Furthermore, the effect of prolactin on amniotic permeability appears unrelated to an osmotic effect by prolactin.<sup>5</sup> Because of the high purity of the ovine prolactin used, the argument that vasopressin contamination can account for membrane permeability changes is no longer acceptable. Even at high concentrations, vasopressin is ineffective in altering amniotic permeability to tritiated water.<sup>3</sup>

Since permeability constants describe rate rather than bulk flow, it is inappropriate to conclude that a reduction in amniotic permeability to tritiated water by prolactin reflects an *in vivo* effect of decidual or amniotic fluid prolactin on net water transfer between fetal and maternal compartments. However, Josimovich et al.<sup>16</sup> and Ross et al.<sup>17</sup> clearly demonstrated an *in vivo* effect of prolactin on amniotic fluid volume in rhesus monkey and sheep preparations, respectively. Thus, although evidence of a prolactin receptor complex in human amnion is lacking, it appears that amnion is biologically responsive to prolactin at the level of the surface epithelium.

The technical assistance of Miss Marsha Leith, Mrs. Agnes Wodzicki, and Mrs. R. Schurath is greatly appreciated. Ovine prolactin was generously provided by the National Hormone and Pituitary Program of the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases.

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## Amniotic fluid collagenase inhibitor: Correlation with gestational age and fetal lung maturity

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Concentrations of collagenase inhibitor in amniotic fluid correlated with gestational age and with indices of fetal lung maturity such as the lecithin/sphingomyelin ratio and the presence of phosphatidylglycerol. Possible sources of amniotic fluid collagenase inhibitor were sought, and a number of tissues and fluids, both maternal and fetal in origin, were found to contain or synthesize this glycoprotein in significant quantities. The highest concentrations were achieved in cultures of lung fibroblasts implicating the fetal lung as a major source. Although collagenase inhibitor is largely present in a free or available state, its specific role, other than that of a general antiproteinase, has not been determined in amniotic fluid. However, the quantitation of amniotic fluid collagenase inhibitor provides an index of the maturation of the connective tissue of the fetal lung and may reflect the ability of the extracellular matrix to meet neonatal demands. (*AM J OBSTET GYNECOL* 1986;154:134-9.)

**Key words:** Collagenase inhibitor, amniotic fluid, collagenase, connective tissue matrix, fetal lung maturity

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The initial and rate-limiting step of collagen degradation in the connective tissue matrix appears to be cleavage by a specific class of proteases, the collagenases (for a recent review, see Reference 1). Control of collagenolysis is crucial to the organism, and regulatory points involving biosynthesis and zymogen activation have been identified with use of human skin procollagenase as a prototypic model of the interstitial collagenases.<sup>1,2</sup> Once collagenase is present in active form within the extracellular space, however, its modulation appears to depend heavily on the action of tissue-de-



rived proteins<sup>2,3</sup> whose inhibitory spectra include other connective tissue metalloproteases such as gelatinase or proteoglycanase.<sup>3</sup> One such inhibitor is secreted by human skin fibroblasts in culture and has been purified and characterized.<sup>4</sup> A survey has revealed the presence of functionally and immunologically identical inhibitors in the media of either cell or explant cultures of lung, bone, cartilage, tendon, cornea, gingiva, and uterine smooth muscle origin. Significant levels are also found in serum and amniotic fluid.<sup>5-7</sup>

With use of a sensitive and specific immunoassay for the detection and quantitation of this protein,<sup>5</sup> we measured levels of amniotic fluid collagenase inhibitor throughout gestation. The data indicate that the concentration of amniotic fluid collagenase inhibitor significantly and positively correlates with gestational age and with standard indices of fetal lung maturity such as the lecithin/sphingomyelin ratio.

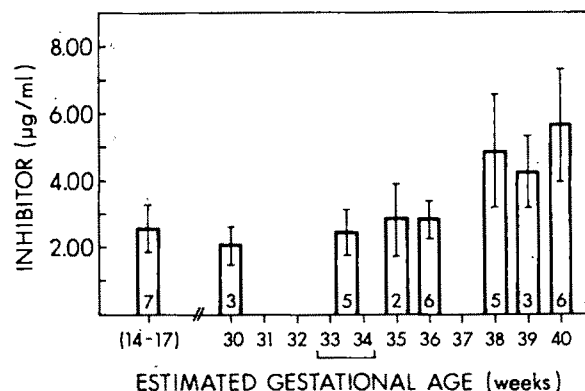
### Material and methods

**Reagents.** Bovine pancreatic trypsin, soybean trypsin inhibitor, goat antirabbit immunoglobulin G-alkaline phosphatase conjugate, phosphatase reagent, and Tris base were purchased from Sigma Chemical Company, St. Louis.

**Patient selection.** The samples of amniotic fluid were obtained during routine amniocenteses performed for either antenatal diagnosis of genetic disorders or the evaluation of fetal lung maturity at the Washington University Perinatal Laboratory. Fluids obtained from patients in whom a genetic disorder was found in the fetus were excluded from this study. All samples were visually free of blood and were spun at  $800 \times g$  for 5 minutes before storage at  $-20^\circ \text{C}$ . Urine of newborn infants represented excess material obtained from routine samples.

**Estimation of gestational age and fetal lung maturity.** The estimated gestational age was determined by patient history and by ultrasonic cephalometry performed by the Perinatal Laboratory, Department of Obstetrics and Gynecology, Washington University School of Medicine. Determination of the lecithin/sphingomyelin ratio and quantitation of phosphatidylglycerol was performed in the clinical laboratory of Dr. Walter G. Weist with use of thin-layer chromatography in a modification of the technique of Morrison and Ruh.<sup>8</sup>

**Cell and explant culture methods.** Normal human fetal skin fibroblasts (AG 4525, AG 4392, AG 4431, GM 0010), normal adult lung fibroblasts (AG 2258, AG 2262), fetal lung fibroblasts (AG 4526, GM 1380, AG 4432, AG 4393), and amniotic fluid cells (GM 0474) were purchased from the Human Genetic Mutant Cell Repository. Another adult lung fibroblast line was



**Fig. 1.** Correlation of amniotic fluid collagenase inhibitor with estimated gestational age. Mean  $\pm$  1 SD is shown for the weeks sampled. The sample size is given by the number written inside the bars. The level of amniotic fluid collagenase inhibitor appears to remain relatively constant until week 38. Statistically, the mean of weeks 14 to 36 ( $2.54 \pm 0.64$ ) is significantly less than that of the mean ( $5.02 \pm 1.57$ ) derived from weeks 38 to 40 ( $p < 0.001$ ). Similarly, the individual means of weeks 38 ( $4.82 \pm 1.69$ ), 39 ( $4.19 \pm 1.05$ ), and 40 ( $5.59 \pm 1.68$ ) differ significantly from the mean of weeks 14 to 36 ( $p < 0.01$ ,  $0.05$ , and  $0.001$ , respectively).

kindly provided by Joan Clark, M.D., Pulmonary Division, Washington University School of Medicine, St. Louis, Missouri, as previously reported.<sup>5</sup> Normal adult skin fibroblasts were purchased from the American Type Culture Collection (CRL 1224), or were initiated from 3 mm punch biopsy specimens of normal skin. Cells were maintained in  $75 \text{ cm}^2$  plastic flasks at  $37^\circ \text{C}$  in Dulbecco's Modified Eagle's Medium-high glucose plus glutamine containing  $0.03 \text{ mol/L}$  HEPES, pH 7.6, 10% fetal bovine serum, and  $200 \text{ U/ml}$  of penicillin and  $200 \text{ µg/ml}$  of streptomycin. Samples of amnion and chorion from uncomplicated term pregnancy were manually separated and minced, then placed in explant culture with use of the medium described above. The medium changed daily for 7 days and stored at  $-20^\circ \text{C}$ .

**Immunoassay of collagenase inhibitor.** Quantitation of inhibitor was performed with use of a sensitive and specific enzyme-linked immunosorbent assay.<sup>5</sup> This assay has been used to measure inhibitor levels in cell or explant culture medium, serum, and amniotic fluid. Levels as determined by enzyme-linked immunosorbent assay agree with those obtained from functional assays.<sup>5</sup> Mic-2000 96-well plates (Dynatech Laboratories, Inc., Alexandria, Virginia) were used, and in the present studies a sensitivity of  $20 \text{ ng/ml}$  was achieved.

**Immunoassay of collagenase.** Human fibroblast collagenase was also quantitated with use of an enzyme-linked immunosorbent assay.<sup>9</sup> Assays of active human skin collagenase alone or in the presence of a fortyfold

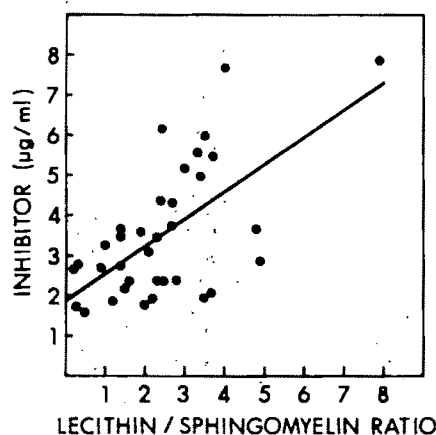


Fig. 2. Correlation of amniotic fluid collagenase inhibitor with the lecithin/sphingomyelin ratio. Linear regression analysis was used to fit these data points and a correlation coefficient of 0.612 was obtained ( $r_{\text{test}} > 99.9\%$ ).

molar excess of purified collagenase inhibitor were performed, and they established that this enzyme-linked immunosorbent assay does not distinguish between free enzyme and that complexed to inhibitor; thus, as was true for the inhibitor enzyme-linked immunosorbent assay,<sup>5</sup> this assay accurately measures total enzyme protein, whether free or bound. Immulon II 96-well plates were used (Dynatech Laboratories) and a sensitivity of 20 ng/ml was achieved.

**High pressure liquid chromatography.** For these studies amniotic fluid was dialyzed for 24 hours against 0.001 mol/L Tris hydrochloride, pH 7.5, lyophilized, and reconstituted in 0.05 mol/L Tris hydrochloride, pH 7.5, in a volume calculated to yield a twentyfold concentration. After clarification, 50  $\mu$ l of this preparation was subjected to gel filtration chromatography with use of an Alltech TSK, type SW guard column coupled to a Bio-Rad TSK-250 column (7.5  $\times$  300 mm) equilibrated in 0.05 mol/L Tris hydrochloride, pH 7.5, containing 0.10 mol/L sodium sulfate and 0.005 mol/L calcium chloride. The pumps, controller, and spectrophotometer were Waters Associates products and were used to maintain a flow rate of 0.8 ml/min (0.5 minute fractions) and to monitor the effluent at 229 nm.

## Results

A constant level of collagenase inhibitor was present in amniotic fluid through the initial 36 weeks of gestation (Fig. 1). During weeks 38, 39, and 40, however, levels gradually increased. These differences were significant when the mean of weeks 14 to 36 was compared to that of weeks 38, 39, or 40 collectively ( $p < 0.001$ ) and individually ( $p < 0.01$ , 0.05, and 0.001, respectively).

Since the concentration of amniotic fluid collagenase inhibitor increased during the last few weeks of ges-

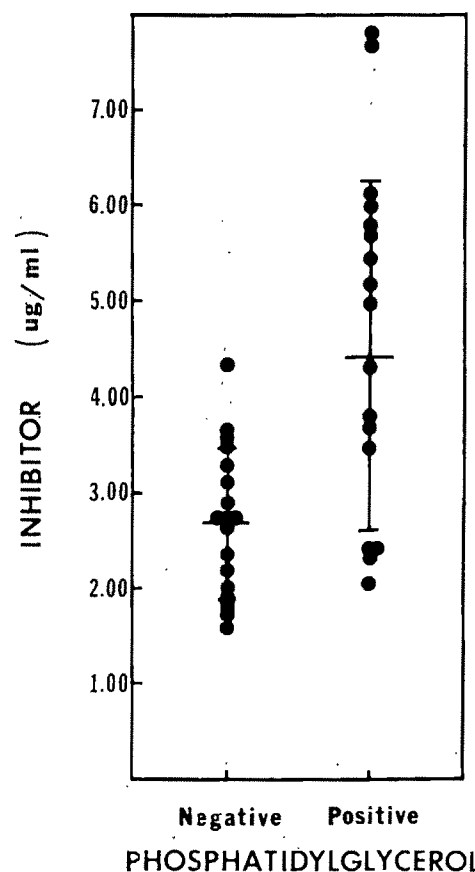


Fig. 3. Correlation of amniotic fluid collagenase inhibitor with phosphatidylglycerol. A scattergram is shown which relates amniotic fluid collagenase inhibitor levels with the absence or presence of phosphatidylglycerol. Means  $\pm$  1 SD are displayed by the solid bars. The values for phosphatidylglycerol (negative =  $2.67 \pm 0.80$ ; positive =  $4.41 \pm 1.84$ ) are significant at  $p < 0.001$ .

tation, a period in which the fetal lungs attain maturity sufficient to avoid the neonatal respiratory distress syndrome,<sup>10</sup> it was of interest to compare inhibitor levels with production of pulmonary surfactant phospholipids commonly used as indices of fetal lung maturity. We evaluated standard fetal lung maturation studies including the lecithin (phosphatidylcholine)/sphingomyelin ratio and the presence of phosphatidylglycerol in amniotic fluid.<sup>11</sup> As seen in Fig. 2, amniotic fluid collagenase inhibitor levels correlated closely with the lecithin/sphingomyelin ratio. Likewise, a comparison of inhibitor levels with the presence or absence of phosphatidylglycerol in the amniotic fluid clearly indicated that elevated inhibitor levels were associated with a positive determination (Fig. 3).

Because these data suggested a relationship between collagenase inhibitor levels in amniotic fluid and fetal lung maturation, a survey of several reasonable and available sources of this protein was undertaken to identify the origin of amniotic fluid collagenase inhib-

**Table I.** Sources of amniotic fluid collagenase inhibitor: Fluid compartments\*

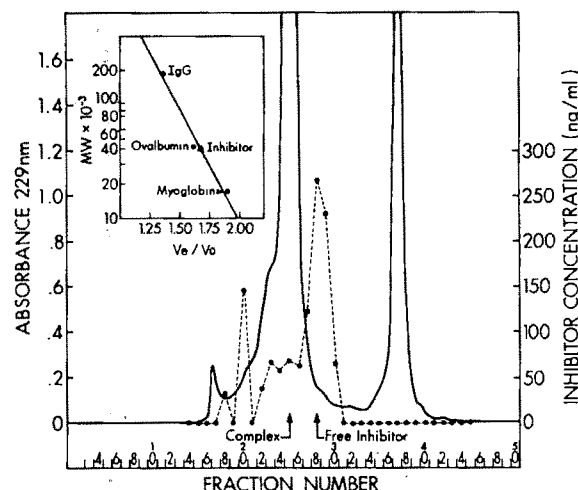
Source	Concentration ( $\mu\text{g/ml}$ )	No. of samples
Maternal serum	$1.06 \pm 0.15$	10
Cord serum	1.15	2
Neonatal urine	$<0.02$	5
Random adult serum†	$1.03 \pm 0.27$	7

\*Immunoreactive collagenase inhibitor was measured directly from neonatal urine and maternal, cord, and random adult sera. Values  $\pm$  1 SD are listed.

†Value as previously reported by Welgus and Stricklin.<sup>5</sup>

itor. Amniotic fluid represents a complex and dynamic mixture whose major constituents include fetal urine, desquamated fetal skin, products sequestered following transplacental passage, and components produced as a result of exchange with the fetal lung. Culture medium obtained from confluent growths of fetal skin fibroblasts, fetal lung fibroblasts, and amniotic fluid cells was assayed for inhibitor as was medium from explant cultures of amnion and chorion. Maternal serum, fetal umbilical cord serum, and neonatal urine were also assayed for the presence of inhibitor. Adult skin and lung fibroblast culture medium and random adult sera were examined for comparative purposes. These data are summarized in Tables I and II. With the sole exception of neonatal urine, appreciable quantities of immunoreactive collagenase inhibitor were found in all of these culture systems and body fluids, both maternal and fetal. Lung fibroblasts, both fetal and adult, produced noticeably higher concentrations of collagenase inhibitor; however, their cell density was also two to three times higher than their dermal counterparts at confluence (data not shown). In this study, no differences in the production of amniotic fluid collagenase inhibitor were noted between the fetal fibroblast lines (12 to 17 fetal weeks) and those obtained from adults.

An assessment was also made of the functional state of amniotic fluid collagenase inhibitor; that is, whether it existed in an unbound, available state or was complexed to metalloproteases such as collagenase.<sup>12</sup> The finding that both functional assays and the inhibitor enzyme-linked immunosorbent assay gave similar values for amniotic fluid collagenase inhibitor concentrations<sup>5</sup> suggested that most of this inhibitor was in an available form. An evaluation of 10 amniotic fluids with use of the collagenase enzyme-linked immunosorbent assay revealed that only a minimal level ( $0.059 \pm 0.027 \mu\text{g/ml}$ ) of immunoreactive collagenase protein was present. As detailed in Material and methods, the collagenase enzyme-linked immunosorbent assay, like its inhibitor counterpart, measures total enzyme, both bound and free; thus, at most,  $<2\%$  of amniotic fluid collagenase inhibitor may be complexed to human fi-



**Fig. 4.** Gel-filtration chromatography of amniotic fluid collagenase inhibitor. A 50  $\mu\text{l}$  aliquot of twentyfold concentrated amniotic fluid was subjected to gel-filtration chromatography as described in text in Material and methods. The absorbance of 229 nm was monitored (—) and the fractions were assayed for amniotic fluid collagenase inhibitor by enzyme-linked immunosorbent assay (• - - - •). Positions of purified inhibitor (free inhibitor) and its complex with collagenase (complex) are indicated.<sup>12</sup> The elution pattern indicates that most of the amniotic fluid collagenase inhibitor is in the free (available) state.

**Table II.** Sources of amniotic fluid collagenase inhibitor: Tissue compartments\*

Tissue	Concentration ( $\mu\text{g/ml}$ )	No. of samples
Fetal skin fibroblasts	$1.38 \pm 0.77$	4
Fetal lung fibroblasts	$6.18 \pm 2.21$	4
Amniotic fluid cells	0.88	1
Amnion explant	1.55	2
Chorion explant	1.30	2
Adult skin fibroblasts	$1.30 \pm 0.11$	4
Adult lung fibroblasts	$6.19 \pm 5.08$	3

\*Medium from confluent cultures of skin and lung fibroblasts as well as amniotic fluid cells was assayed in addition to medium obtained from explant cultures of amnion and chorion. It should be noted that the fetal skin and lung cell lines form four matched pairs; i.e., each skin line has a lung counterpart derived from the same fetus. Values for adult skin and lung fibroblasts are included for comparative purposes. Values  $\pm$  1 SD are listed.

broblast collagenase. Finally, amniotic fluid was subjected to gel filtration chromatography with use of columns and conditions that have been shown to discriminate between free and complexed inhibitor.<sup>12</sup> As seen in Fig. 4, the bulk of the inhibitor eluted at the same position as purified skin fibroblast collagenase inhibitor. About a third of the total immunoreactive amniotic fluid collagenase inhibitor was also found in earlier fractions, indicating that at least some of this inhibitor was either complexed to proteases such as collagenase, or alternatively, was present in aggregated form.

### Comment

Human skin fibroblasts secrete a 28,500 dalton glycoprotein that is capable of inhibiting human skin collagenase as well as other mammalian collagenases. The spectrum of inhibition extends to several other neutral proteases of connective tissue origin such as human skin explant gelatinase and a neutral protease secreted by skin fibroblasts in tissue culture.<sup>2</sup> Other investigators have described similar collagenase inhibitors from a number of human and animal sources, and it has been postulated that the function of these proteins is to inhibit neutral metalloproteases of connective tissue origin.<sup>3</sup> In the human, immunologically and functionally identical inhibitor proteins have been found in most connective tissues, in serum, and in amniotic fluid.<sup>5-7</sup> This almost ubiquitous presence suggests that this inhibitor may play a very basic and crucial role in the regulation of collagenolysis and matrix turnover. By quantitating the levels of collagenase inhibitor in amniotic fluid we have begun the process of establishing its origin and function. Our data indicate that the levels of amniotic fluid collagenase inhibitor correlate significantly with gestational age as well as with indices of fetal lung maturity such as the lecithin/sphingomyelin ratio and the presence of phosphatidylglycerol.

We were unable to definitely determine the origin of amniotic fluid collagenase inhibitor. Cell density studies with use of dermal fibroblasts demonstrate that the accumulation of this protein in culture medium reaches a fairly stable plateau,<sup>13</sup> which may be essentially independent of cell number. Thus the higher concentrations achieved by lung fibroblasts may, in fact, be significant. It is appropriate, therefore, to speculate that the concentration of amniotic fluid collagenase inhibitor may be a marker of fetal lung maturity from a connective tissue viewpoint in much the same way as the lecithin/sphingomyelin ratio or the presence of phosphatidylglycerol is a marker of lung surfactant metabolism.<sup>10, 11</sup> Alternatively, one or more of the other sources of this protein may be responsible for its appearance in the amniotic fluid, or a trapping mechanism may cause the observed levels.

A specific role for amniotic fluid collagenase inhibitor in the amniotic fluid compartment has not been established. A similar, if not identical, collagenase inhibitor appears to be almost ubiquitous in the human organism and may serve a basic role in limiting the action of collagenases and other degradative enzymes of connective tissue origin.<sup>5</sup> This protein may function as a general inhibitor of collagen degradation in the amniotic fluid in much the same fashion as  $\alpha_2$ -macroglobulin does in the plasma.<sup>14</sup> In this regard, a calculation of the relative contribution to inhibition represented by  $\alpha_2$ -macroglobulin (1.67  $\mu$ g/ml, molecular weight of 720,000, Reference 15) and amniotic fluid collagenase

inhibitor (3  $\mu$ g/ml, molecular weight of 28,500) revealed that >95% of collagenase inhibitory activity in this compartment would be expected to arise from the action of the smaller molecular weight protein. Furthermore, both by measures of functional activity<sup>5</sup> and by its distribution following gel-filtration chromatography, it appears that most of the amniotic fluid collagenase inhibitor exists in an unbound state.

Two developmental changes occur in the fetal lung near term which appear to be primarily responsible for allowing the neonate to avoid respiratory compromise following birth. The first of these changes occurs in the connective tissue framework of the lung and involves changes in pulmonary compliance which permit proper lung expansion.<sup>16</sup> The second, and better known, involves sequential changes in the phospholipid content and composition of pulmonary surfactant in the developing fetal lung. Surfactant exchange with amniotic fluid produces characteristic changes in the ratio of dipalmitoyl phosphatidylcholine (lecithin) to sphingomyelin<sup>10</sup> and in the appearance of phosphatidylglycerol in the amniotic fluid; these changes usually begin by 36 weeks of gestation.<sup>11</sup> Over the last decade these measures of the amniotic fluid phospholipid profile have become the standard for evaluation of fetal lung maturity. It may well be that amniotic fluid collagenase inhibitor levels provide us with the first biochemical index of maturational change within the fetal lung collagen matrix. This index may reflect changes in the ability of the developing lung to meet the demands of pulmonary expansion in the neonatal period.

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## Effect of weight loss and antiandrogenic therapy on sex hormone blood levels and insulin resistance in obese patients with polycystic ovaries

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This study was performed in two randomly defined groups of obese patients with polycystic ovaries to investigate the overall effects of hypocaloric diet combined (group 2) or not combined (group 1) with an antiandrogenic therapy (cyproterone acetate, 50 mg/day, plus ethinyl estradiol, 0.05 mg/day) on sex hormone plasma levels, insulin secretion and resistance, and body weight loss and on their reciprocal interrelationships. All obese patients with polycystic ovaries showed elevated luteinizing hormone and androgen levels, hyperinsulinemia, and marked insulin resistance. After an average period of 3 months both groups showed a similar weight loss and a similar reduction in the insulin-resistant state. During treatment in group 1 three patients had a greater frequency of menstrual bleeding, and in one of them an ovulatory cycle was documented. Whereas, no changes in gonadotropin and sex steroid levels were found in group 1, a significant fall was observed in group 2. No relationships were observed between these changes and those which occurred on insulin levels. We conclude that hyperandrogenism in obese patients with polycystic ovaries does not appear to be a primary factor leading to the insulin-resistant state. (*AM J OBSTET GYNECOL* 1986;154:139-44.)

**Key words:** Ovarian polycystic disease, obesity, androgens, insulin resistance, weight loss

Hyperinsulinemia and insulin resistance represent common features in both obese<sup>1, 2</sup> and normal-weight<sup>2, 3</sup> patients with polycystic ovaries. A correlation between hyperinsulinemia and hyperandrogenism has been demonstrated repeatedly,<sup>1-4</sup> but the mechanisms

underlying this relationship remain obscure. Various hypotheses have been developed: several authors<sup>1</sup> and our group<sup>2</sup> have suggested that hyperandrogenism could be a primary event leading to insulin resistance, whereas other investigators<sup>4, 5</sup> have speculated that insulin resistance may be partially responsible for the excessive androgen synthesis. What the effects of a marked and prolonged suppression of hyperandrogenism may be on insulin resistance in women with polycystic ovaries has not yet been investigated to a sufficient extent. Reduction of body weight by hypocaloric dietary treatment is the principal means of improving hyperinsulinemia and insulin resistance in patients with

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**Table I.** General data of the obese patients with polycystic ovaries participating in this study

<i>Patients</i>	<i>Age (yr)</i>	<i>Body weight (kg)</i>	<i>Body mass index</i>	<i>Age at menarche (yr)</i>	<i>Age at onset of obesity (yr)</i>
Group 1					
P. A.	17	88.9	29.7	13	7
M. M.	25	75.0	30.5	14	14
F. A.	19	71.4	33.5	14	12
L. K.	23	84.0	31.2	12	7
G. D.	26	93.0	34.2	14	18
T. E.	25	92.6	34.0	13	14
M. R.	27	99.5	41.4	13	22
Mean	23.1	86.6	33.5	13.3	13.4
SE	1.4	3.8	1.5	0.3	2.1
Group 2					
T. M.	21	88.0	35.8	12	20
C. A.	23	77.5	29.1	17	14
D. E.	18	80.3	28.2	14	16
T. P.	27	86.0	30.5	11	7
B. S.	18	84.0	35.4	13	9
C. L.	21	93.0	33.1	13	8
V. P.	19	77.5	31.9	10	7
Mean	21.0	83.8	32.0	12.8	11.5
SE	1.2	2.2	1.1	0.8	1.9
Significance	NS	NS	NS	NS	NS

simple obesity; moreover, several studies performed on ovulatory and anovulatory obese women<sup>6-8</sup> have shown that weight loss can partially correct gonadotropin and sex steroid abnormalities and frequently reestablish a normal condition of fertility. However, therapy with cyproterone acetate combined with ethinyl estradiol has been used for many years in the treatment of patients with polycystic ovaries, since it possesses antiandrogenic properties by inhibiting gonadotropin levels and excessive gonadal androgen production.<sup>9</sup> Studies performed some years ago showed that prolonged treatments with this therapeutic regimen seem not to alter glucose tolerance and insulin secretion in man.<sup>10</sup> With this background we performed our study in a sample of obese women with polycystic ovaries to investigate the overall effects of hypocaloric diet combined or not combined with the above-mentioned antiandrogenic regimen on gonadotropin and sex steroid plasma levels, glucose tolerance, insulin values, and body weight and on their reciprocal interrelationships.

#### Material and methods

**Patients and protocol study.** Fourteen informed female patients affected by polycystic ovaries and obesity (body mass index, >28) were included in the study. Before entry into the study, a diagnosis of polycystic ovaries was made based on the presence of oligomenorrhea or amenorrhea, hirsutism, hyperandrogenism, elevated luteinizing hormone/follicle-stimulating hormone ratio, ultrasound evaluation of the ovaries, and in some patients, because of laparoscopy and histologic

findings as previously described.<sup>2</sup> Physical examination showed that two women had acanthosis nigricans localized on the nape. Spontaneous caloric intake, which was evaluated for the majority of patients by means of interviews (dietary history) performed by a well-trained dietician and by a 3-day record technique, was  $2344 \pm 347$  kcal/day in group 1 (five observations) and  $2026 \pm 148$  kcal/day in group 2 (six observations) (p, not significant). Age of menarche and age of onset of oligomenorrhea or amenorrhea and of obesity were carefully recorded. Before the testing all patients followed a 3-day, 2200 kcal weight-maintenance diet containing 250 gm of carbohydrates. Hematologic examinations were performed in the morning (8:00 to 8:30 AM) after an overnight fast. Blood samples for hormonal determination were obtained in the fasting during the early follicular phase in women with oligomenorrhea and in a period chosen at random in those presenting with amenorrhea lasting more than 3 months. An oral glucose (Curvoso, Sclavo, Italy) tolerance test was then performed (1gm/per kilogram of body weight): Blood samples were obtained at 0, 30, 60, 90, 120, and 180 minutes for glucose and at 0, 60, 120, and 180 minutes for insulin and C-peptide determination.

After all baseline examinations had been completed, all patients followed the therapeutic program that consisted of a hypocaloric diet of 1000 or 1200 kcal/day (containing 20% protein, 30% lipids, and 50% carbohydrates) combined (group 2) or not combined (group 1) with a reversed sequential regimen with cyproterone acetate (Androcur, Schering, Milan, Italy) and ethinyl

estradiol (Ethinilestradiolo, Samil, Rome, Italy). Starting on the fifth day after the onset of menstrual bleeding in patients with oligomenorrhea and in a random period in those presenting with amenorrhea, 50 mg of cyproterone acetate were given daily for 10 days combined with 0.05 mg of ethinyl estradiol for 21 days, followed by an interval of 7 days. The choice of treatment for each patient was randomized. Each group consisted of seven patients, matched for age, body weight, body mass index and spontaneous caloric intake (mean values were 1100 kcal/day in group 1 and 1056 kcal/day in group 2; *p*, not significant); coincidentally, women with acanthosis nigricans were equally distributed in the two groups. General data of all obese patients with polycystic ovaries are summarized in Table I.

Clinical conditions, weight loss, and adherence to the diet and to pharmacologic treatment were evaluated every 3 or 4 weeks. After an average period of 3 months all patients were subjected, under the same experimental conditions, to a second complete clinical and laboratory examination, which included basal hormonal determinations and an oral glucose tolerance test (performed by administering the same quantity of glucose). Before reexamination every woman again followed a 3-day diet of 2200 kcal/day, containing 250/gm of carbohydrates. For the women from group 2 the clinical data and blood samples were collected while they were still taking hormone therapy, whereas for the majority of patients from group 1 the data and samples were obtained in the early follicular phase, except for one patient who was evaluated in the late luteal phase.

**Hormonal and biochemical measurements.** Blood glucose level was determined immediately after the end of the oral glucose tolerance test by the glucose-oxidase method, whereas hormone assays were performed on serum or plasma samples stored at  $-20^{\circ}\text{C}$  until analysis. All hormone determinations in samples collected before and after weight loss from each patient were performed in the same assay. Insulin levels were determined by bovine antiinsulin antiserum fixed on glass particles with a kit supplied by Corning Medical Diagnostic (Medfield, Massachusetts) and C-peptide was determined using reagents supplied by Byk-Mallinckrodt (Milan, Italy). Gonadotropin (follicle-stimulating hormone and luteinizing hormone; standard: second International Reference Preparation human menopausal gonadotropin) and prolactin plasma levels were determined with kits purchased from Biodata (Rome, Italy);  $17\alpha$ -hydroxyprogesterone and progesterone by chromatographic separation on Sephadex LH-20 columns; dehydroepiandrosterone sulphate directly on diluted plasma; testosterone,  $5\alpha$ -dihydrotestosterone, androstenedione, estrone, estradiol- $17\beta$  by thin-layer chromatogram on silica gel 60F254, using antisera

**Table II.** Duration of the study and changes in obesity index in the two groups of obese patients with polycystic ovaries evaluated in this study

Patients	Duration of study period (days)	Body mass index		Change in body mass index (%)
		Initial	Final	
Group 1				
P. A.	95	29.7	26.4	-11.2
M. M.	115	30.5	28.0	-8.2
F. A.	93	33.5	31.9	-4.8
L. K.	90	31.2	27.1	-13.2
G. D.	83	34.2	33.0	-3.5
T. E.	100	34.0	30.9	-9.8
M. R.	90	41.4	37.7	-9.0
Mean	95.1	33.5	30.7	-8.5
SE	3.8	1.5	1.5	1.3
Group 2				
T. M.	100	35.8	32.8	-8.4
C. A.	105	29.1	25.7	-11.7
D. E.	83	28.2	25.0	-11.4
T. P.	90	30.5	27.6	-9.6
B. S.	104	35.4	33.7	-4.2
C. L.	90	33.1	31.9	-3.7
V. P.	180	31.9	24.7	-22.6
Mean	107.4	32.0	28.8	-10.2*
SE	12.5	1.1	1.5	2.4
Significance	NS	NS	NS	NS

\*Mean value of percent change in body mass index in group 2 was  $8.2 \pm 1.2$  when values of body mass index after 3 months of treatment in Patient V. P. were excluded.

made in our laboratory. All procedures followed for hormone determination have been reported previously.<sup>2</sup>

Statistics were performed using the Student's *t* test for paired (when comparison between changes of each parameter before and after treatment in each separate group was performed) and unpaired (when comparison between the two groups was performed) data and the Pearson's regression analysis. Results are reported as mean  $\pm$  standard error of the mean.

For comparison, basal gonadotropin and sex steroid plasma patterns in 19 fertile control subjects of normal weight are also reported. Fasting and glucose-stimulated values of glucose, insulin, and C-peptide were obtained from 12 of them. All examinations were performed in accordance with the procedure described above and were carried out during the early follicular phase (days 4 to 6 of the cycle).

## Results

All patients complied well with the dietary and pharmacologic treatment. The individual prescribed diets, the duration of the period of observation, and changes in body weight are reported in Table II: no significant differences were present between the two groups. In

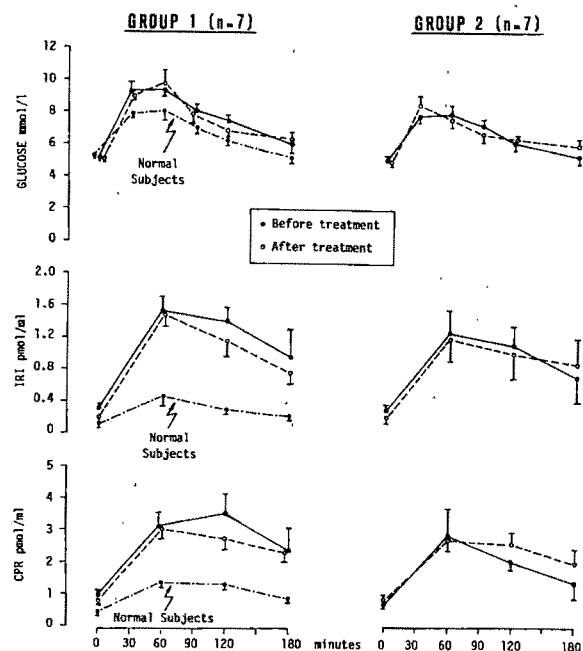


Fig. 1. Glucose, insulin (IRI), and C-peptide (CPR) blood values in fasting conditions and during oral glucose tolerance test in the two groups of obese patients with polycystic ovaries before and after each therapeutic program (see text). In the left panel, values from 12 control subjects with normal weight are also reported (○—○).

group 1 at least three patients (P. A., T. E., and M. R.) showed a greater frequency of spontaneous menstrual bleeding than before, and an ovulatory cycle (confirmed by the demonstration of elevated progesterone levels) occurred in Patient P. A. after she had been amenorrheic for 10 months before this study. An increased loss of excessive body hair occurred in four patients from groups 2, whereas no evident variation was observed in patients from group 1.

Pretreatment fasting and glucose-stimulated values of glucose, insulin and C-peptide are reported in Fig. 1. Three patients in group 1 (M. N., L. K., and M. R.) and one in group 2 (V. P.) showed impaired glucose tolerance according to the National Diabetes Data Group Criteria (1979). Nevertheless, mean glucose values were practically the same as those of the control group, whereas significantly different glucose levels at 60 ( $p < 0.05$ ) and 120 ( $p < 0.05$ ) minutes during oral glucose tolerance tests were present between the two groups. Compared to controls, all obese patients with polycystic ovaries presented conversely elevated values of insulin and C-peptide in fasting conditions and during oral glucose tolerance tests, without differences between the two groups. Treatment failed to induce any significant variation in glucose, insulin, and C-peptide absolute blood levels, but differences in glucose values at 60 and 120 minutes during oral glucose tolerance tests between the two groups disappeared. Fig. 2 re-

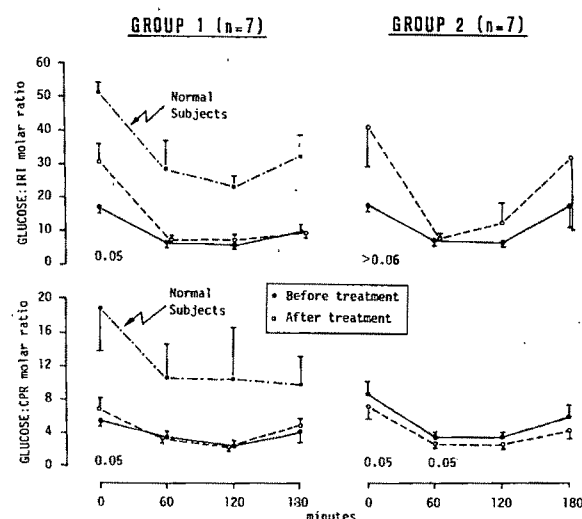


Fig. 2. Glucose/insulin and glucose/C-peptide molar ratios during oral glucose tolerance test in the two groups of obese patients with polycystic ovaries evaluated in this study before and after each therapeutic program (see text). In the left panel, values from 12 control subjects with normal weight are also reported (○—○).

ports the glucose/insulin and the glucose/C-peptide molar ratios, which can be considered, respectively, as reliable indexes of peripheral insulin sensitivity (and therefore of insulin resistance) and  $\beta$  cell secretion capacity. Fasting values increased significantly to a similar extent in both groups, without appreciable modifications in the values during the oral glucose tolerance test.

Gonadotropin, prolactin, and steroid plasma concentrations before and after therapy are reported in Table III. Pretreatment values in both groups were practically the same. In group 1, in spite of five patients showing an evident reduction in testosterone levels, diet therapy was not followed by significant changes in any of the hormones. It must however be pointed out that Patient P. A. was excluded from the analysis of these data, having been reevaluated during the luteal phase. Conversely, in group 2 a significant fall occurred in plasma gonadotropin and steroid levels as an obvious consequence of the antiandrogenic therapy. We failed to observe in either group any significant correlation between hormonal variations, the amount of body weight loss, and changes in the fasting or glucose-stimulated insulin and C-peptide levels.

With all obese patients with polycystic ovaries considered together, we also analyzed the relationships existing between pretreatment values of body mass index and fasting and stimulated insulin and C-peptide and those of sex hormones. Body mass index showed a significant positive correlation with luteinizing hormone ( $r = 0.52$ ,  $p < 0.01$ ) and estrone ( $r = 0.78$ ,  $p < 0.01$ ) levels. Testosterone values were significantly correlated



**Table III.** Gonadotropin and sex steroid pattern before and after treatment in the two groups of obese patients with polycystic ovaries evaluated in this study

Hormone	Group 1*		Group 2		Control group
	Before (n = 7)	After (n = 6)	Before (n = 7)	After (n = 7)	
Luteinizing hormone (mU/ml)	18.4 ± 2.2	18.6 ± 3.1	20.7 ± 3.5	7.7 ± 2.1†	9.0 ± 0.6
Follicle-stimulating hormone (mU/ml)	8.3 ± 0.5	8.7 ± 0.8	7.8 ± 0.3	4.4 ± 1.2†	10.0 ± 0.4
Luteinizing hormone/ follicle-stimulating hormone ratio	2.28 ± 0.35	2.13 ± 0.30	2.80 ± 0.61	1.84 ± 0.34†	1.0 ± 0.2
Prolactin (ng/ml)	7.4 ± 1.3	7.6 ± 1.4	10.0 ± 2.7	17.5 ± 3.4	12.9 ± 2.0
Estradiol (pg/ml)	39.5 ± 11.0	41.0 ± 1.4	31.9 ± 6.0	11.6 ± 1.0†	32.8 ± 2.2
Estrone (pg/ml)	52.2 ± 8.2	45.7 ± 5.3	51.1 ± 8.4	21.7 ± 3.6‡	32.9 ± 2.7
Testosterone (ng/dl)	71.3 ± 14.4	59.0 ± 11.7	63.4 ± 14.1	29.8 ± 2.6†	26.6 ± 1.6
Androstenedione (ng/dl)	266.5 ± 40.4	305.2 ± 79.0	329.4 ± 51.3	159.8 ± 28.7†	138.0 ± 5.8
5 $\alpha$ -Dihydrotestosterone (ng/dl)	23.3 ± 5.8	23.3 ± 5.6	19.8 ± 2.2	15.0 ± 1.4†	17.1 ± 1.6
Dehydroepiandrosterone sulfate ( $\mu$ g/dl)	3.38 ± 0.61	3.20 ± 0.30	2.63 ± 0.29	2.05 ± 0.28†	2.50 ± 0.21
Dehydroepiandrosterone (ng/ml)	8.84 ± 1.05	9.27 ± 0.90	9.03 ± 1.51	5.74 ± 1.16†	5.30 ± 0.40
17 $\alpha$ -Hydroxyprogesterone (ng/ml)	0.80 ± 0.10	0.75 ± 0.11	0.94 ± 0.15	0.65 ± 0.07†	0.51 ± 0.02
Progesterone (ng/ml)	0.22 ± 0.04	0.23 ± 0.03	0.24 ± 0.05	0.17 ± 0.03	0.22 ± 0.02
Estradiol/estrone ratio	0.67 ± 0.11	0.91 ± 0.09†	0.66 ± 0.08	0.58 ± 0.06	0.99 ± 0.05

\*Results of the treatment in group 1 refer to six patients only (see text for explanation).

†p < 0.05.

‡p < 0.01.

with fasting insulin ( $r = 0.46$ ,  $p < 0.05$ ) and the sum of insulin ( $r = 0.54$ ,  $p < 0.05$ ) and C-peptide levels ( $r = 0.51$ ,  $p < 0.01$ ) during oral glucose tolerance tests. Finally, a significant correlation coefficient between plasma androstenedione and the sum of insulin values during oral glucose tolerance tests was found ( $r = 0.45$ ,  $p < 0.05$ ).

### Comment

In basal conditions, all obese patients with polycystic ovaries presented marked and increased  $\beta$  cell activity associated with normal or moderately impaired glucose tolerance. Moreover, similarly to what was previously reported by several groups<sup>1,3,4</sup> and by us,<sup>2</sup> a significant correlation was found between hyperinsulinemia and plasma androgen levels. All of these relationships appeared to be relatively independent of the degree of excess body weight.

Dietary treatment induced a similar weight loss, both in patients treated with diet alone (group 1) and in those in whom the antiandrogenic therapy was combined (group 2); in both groups a similar improvement of insulin resistance, at least in the fasting state, was observed irrespective of pharmacologic therapy.

Absolute mean values of fasting gonadotropin and sex steroid plasma levels did not present any significant variation during treatment in group 1. In reality, a tendency to lowered testosterone levels was observed in several patients from group 1, but because of the wide range of variability, the differences with respect to pretreatment values were not statistically significant. On the contrary, the estradiol/estrone molar ratio significantly increased after weight loss, nearly reaching

the normal range. Our study does not therefore confirm completely what was observed by other authors with regard to testosterone levels in obese anovulatory<sup>6,7</sup> and obese women with polycystic ovaries.<sup>8</sup> The measurement of free testosterone would probably have solved these discrepancies. However, the sex hormone/binding hormone levels, which tend to be reduced when obesity is present, have been found to be significantly increased after weight reduction in anovulatory obese women.<sup>7</sup> These findings suggest that in group 1 we would probably have been able to observe a reduction also in free testosterone in agreement with what was partly observed for testosterone. The estradiol/estrone ratio, on the contrary, was significantly increased by weight loss in group 1, but no changes in the luteinizing hormone plasma concentrations were observed. These results are in agreement with what was observed in testosterone levels but do not agree with the hypothesis that the luteinizing hormone hypersecretion can be a primary factor in determining ovarian steroidogenesis irregularities in all obese patients who have polycystic ovaries. Small changes in steroid metabolism as a consequence of weight loss probably justify the occurrence of more frequent menstrual cycles (and the demonstration of an ovulatory cycle) observed in some patients in group 1. Thus, as can also be assumed from other studies,<sup>7,8</sup> it is possible to hypothesize that weight loss does however represent an important therapeutic measure in obese women with polycystic ovaries.

Hormonal treatment in group 2 induced significantly lower basal gonadotropin and androgen plasma levels, as was expected on the basis of previous reports.<sup>9</sup> On

the other hand, these changes did not correlate with those observed in insulin resistance and insulin secretion. It is improbable that these results were adulterated by the administration of the cyproterone and ethinyl estradiol therapy, since so far it has been shown to have no effect on insulin secretion and glucose tolerance<sup>10</sup> or on body weight.<sup>10</sup> Moreover, studies on tissue culture of mouse pancreatic islets have shown that ethinyl estradiol has no effect on insulin content and release.<sup>11</sup> Contrary to what was previously suggested by others<sup>1</sup> and by us,<sup>2</sup> these findings do not seem to support the hypothesis that peripheral androgen metabolism does play a major role in the development of hyperinsulinemia and insulin resistance, in spite of the reciprocal relationship detectable in their peripheral blood concentrations. Conversely, it could be suggested that obesity as such may represent an important factor both in the genesis of insulin resistance and in that of hyperandrogenism in these patients.<sup>4</sup> This hypothesis does not explain, however, why women of normal weight with polycystic ovaries may present a condition of insulin resistance.<sup>2,3</sup> A common causative mechanism could be represented by the high levels of plasma  $\beta$ -endorphin, which has been found to be significantly elevated in obese and normal-weight patients with polycystic ovaries<sup>12</sup> as well as being positively correlated with body weight.<sup>12</sup> In fact,  $\beta$ -endorphin has the capacity to stimulate insulin secretion in man.<sup>13</sup>

Finally, it should be emphasised that high-affinity insulin receptors have been demonstrated very recently, in ovaries from women with<sup>14</sup> or without<sup>15</sup> polycystic ovaries. One interesting hypothesis that follows is that some disease states of polycystic ovaries could result from hyperinsulinemic obesity. Preliminary studies performed on porcine granulosa cell monolayers have demonstrated that insulin is critical for the maintenance of their several functional properties<sup>16</sup> and others have shown that it may be a regulator of steroidogenesis in cultured porcine thecal cells.<sup>5</sup>

In conclusion, our study confirms that obese patients with polycystic ovaries present abnormally high fasting and glucose-stimulated insulin levels that are correlated with androgen levels; on the other hand, it suggests that hyperandrogenism does not seem to be a primary causative factor leading to the insulin-resistant state in these patients and indicates the possibility that insulin may play a role in the development of increased androgen production.

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# Toxic shock syndrome following carbon dioxide laser treatment of genital tract condyloma acuminatum

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Toxic shock syndrome was observed in the postoperative period in a patient who underwent carbon dioxide laser treatment of genital condyloma acuminatum. Laser vaporization of the vagina produces areas of denuded mucosa. These areas may act as the portal of entry for staphylococcal toxins producing toxic shock syndrome. (AM J OBSTET GYNECOL 1986;154:145-6.)

**Key words:** Toxic shock syndrome, carbon dioxide laser

Toxic shock syndrome is a multisystem acute illness that primarily affects menstruating women. From 5% to 13% of reported cases of toxic shock syndrome are associated with nonmenstrual medical, surgical, obstetric, and gynecologic conditions.<sup>1</sup> We report a case that followed carbon dioxide laser treatment of genital condyloma acuminatum in a nonmenstruating woman.

## Case report

J. M., a 24-year-old, white woman, para 0-0-1-0, underwent cervical conization, partial vulvectomy (50%), partial vaginectomy (40%), and vaporization of anal condylomas with carbon dioxide laser for treatment of vulvovaginal, cervical, and anal condylomas. There were no intraoperative or immediate postoperative complications. She took sitz baths with Instant Ocean and applied nitrofurazone cream and 2% lidocaine jelly to the affected areas four times a day. No tampons were used.

She became febrile with a temperature of 103° F 24 hours after operation. During the next 24 hours she experienced nausea, vomiting, diarrhea, diffuse muscle aches, sore throat, headache, lightheadedness, and dizziness on standing. The following day she noted chills, sweats, and temperature intermittently to 105° F. Because of continued symptoms and persistent fever above 102° F she returned to be evaluated on the fourth postoperative day.

The last menstrual period had been approximately 3½ weeks earlier. She was using oral contraceptives and was expecting menses within a few days, but menses did not begin until the sixth postoperative day. She had no history of significant medical illnesses.

Examination revealed an acutely ill, flushed woman in some distress. Blood pressure was 100/60 mm Hg and pulse 114 bpm, while supine, with blood pressure 110/70 mm Hg and pulse 134 bpm while sitting. Temperature was 99.9° F and respirations 20/min. The skin was erupted in a generalized macular, erythematous, blanching, nonpruritic rash. The skin had a "sunburn-like" appearance that involved the face, arms, thighs, legs, and trunk. The tongue was "beefy red"; the pharynx was erythematous and the mucous membranes dry. The conjunctivae were injected bilaterally and there was mild scleral icterus. The abdominal right upper quadrant was moderately tender, particularly the liver edge. There was no hepatomegaly or other abdominal abnormality. The pelvic examination revealed multiple denuded areas of the vulva, vagina, and cervix (as expected after laser treatment) with edema, erythema, and a foul purulent vaginal discharge.

The patient was admitted to the hospital for hydration and further evaluation. The white blood cell count was 12,500/mm<sup>3</sup> with an extreme left shift. The hematocrit was 35.7% and platelets 152,000/mm<sup>3</sup>. Coagulation studies were normal; the sedimentation rate was elevated, and urinalysis showed hemoglobin without red blood cells. Hyperbilirubinemia, hypoalbuminemia, hypocalcemia, and elevated liver function tests (transaminases and alkaline phosphatase) were seen. Creatine phosphokinase was normal. Hepatitis B surface antigen was not present.

The patient's vital signs remained stable and the rash and fever resolved within 48 hours. Urine output was always adequate. An ultrasound examination of the liver and gallbladder was normal. Vaginal, rectal, and pharyngeal cultures were positive for *Staphylococcus aureus*.

Two weeks after operation an outpatient evaluation revealed desquamation of the skin of the left axilla and the palmar surface of the fingers. A biopsy was obtained from a desquamating finger. The vulva and vagina were healing well and the purulent discharge had resolved. All abnormal laboratory values had returned to normal or were improving, except the platelet count and the liver transaminase levels, which were elevated. These

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later returned to normal. Over the next 2 weeks there was extensive peeling of the palms and soles.

### Comment

This case fulfills the requirements of the Centers for Disease Control case definition for toxic shock syndrome except for documentation of hypotension.<sup>2</sup> The patient had experienced orthostatic dizziness and had significant pulse elevation on sitting but no drop in blood pressure. Fever, rash, and desquamation were noted as well as involvement of gastrointestinal, mucous membrane, and hepatic systems. Muscular involvement was suggested by the myalgias but the creatine phosphokinase was normal.

The positive pharyngeal culture might suggest staphylococcal scarlet fever as a cause of this patient's illness. The differentiation between this and toxic shock syndrome is made pathologically by the level of separation of the desquamating skin. Biopsy of the skin in this

patient did not distinguish between these two entities. However, the clinical course along with the positive vaginal cultures would favor toxic shock syndrome.

Approximately 9% of women of reproductive age are colonized with *S. aureus* in the vagina or cervix.<sup>1</sup> It has been proposed that microulcerations caused by tampon insertion might act as the portal of entry for the toxin. In this case, ulcerations were produced by the laser in a susceptible patient. Physicians performing laser vaginectomy should be aware of the possibility of toxic shock syndrome as a postoperative complication. The systemic manifestations should be recognized early to avoid potential morbidity or mortality.

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## The relationship between abortion in the first pregnancy and development of pregnancy-induced hypertension in the subsequent pregnancy

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The relation between pregnancy-induced hypertension and reproductive history was assessed in 29,484 women receiving obstetric care at Parkland Memorial Hospital. The incidence of pregnancy-induced hypertension was 25.4% in primigravid women, somewhat lower (22.3%) in women whose only previous pregnancy terminated in abortion, and much lower (10%) in women who carried two or more successive pregnancies to viability. (AM J OBSTET GYNECOL 1986;154:146-8.)

**Key words:** Gestational hypertension, abortion, parity

The etiology of pregnancy-induced hypertension (gestational hypertension) is an enigma. Although many theories have been proposed, some clinical and pathologic observations are consistent with the view

that pregnancy-induced hypertension may be, at least in part, the consequence of an altered immunologic status.<sup>1</sup> One of the most significant of these observations is that a completed first pregnancy confers protection in a second pregnancy.<sup>2</sup> Moreover, MacGillivray<sup>3</sup> has suggested that *any* first pregnancy, even one ending in abortion, affords protection against pregnancy-induced hypertension in the second pregnancy. In a study of 516 women with a completed pregnancy preceded by one abortion ("virtua" primigravidas), he reported an incidence of preeclampsia of 15.7%, as compared with an incidence of 22.2% in "true" primigravidas. By contrast, true secundigravidas had an incidence of pre-

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**Table I.** Relation between reproductive history and pregnancy-induced hypertension

Pregnancy-induced hypertension	Group*							
	1		2		3		4	
	n	%	n	%	n	%	n	%
Present	2503	25.4	285	22.3	589	10.2	743	9.6
Absent	7363	74.6	991	77.7	5162	89.8	7010	90.4
Total	9866		1276		5751		7753	

\*Characteristics of the groups were as follows: 1—gravida 1, para 1; 2—gravida 2, para 1, abortion 1; 3—gravida 2, para 2; 4—gravida  $\geq 3$ .

eclampsia of only 8.0%. Thus an abortion did not appear to confer the degree of protection afforded by a completed first pregnancy.

To our knowledge, MacGillivray's observations have not been confirmed. The present study, therefore, was conducted to assess the relation between pregnancy-induced hypertension and reproductive history in women receiving obstetric care at Parkland Memorial Hospital and clinics during the 40-month period from September, 1977, through December, 1980.

Obstetric data were accessed via a computer record system designated MANDATE (Maternal And Neonatal Data Acquisition Transmission and Evaluation). Error rates of data input and extraction were less than 0.5%.<sup>1</sup>

The diagnosis of pregnancy-induced hypertension was made on the basis of either a blood pressure of 140/90 mm Hg or greater after 20 weeks' gestation or an increase of 30 torr systolic or 15 torr diastolic over baseline blood pressures (that is, those obtained before 20 weeks' gestation).<sup>2</sup> These observations must have been made on at least two occasions 6 or more hours apart.

Women were excluded from analysis for the following reasons: (1) a multiple gestation in the current pregnancy; (2) chronic hypertension, defined as a blood pressure of 140/90 mm Hg or greater either occurring before the twentieth week of the current pregnancy or documented when the woman was not pregnant; (3) maternal renal disease other than uncomplicated cystitis or pyelonephritis, including glomerulonephritis, chronic pyelonephritis, nephrotic syndrome, or renal insufficiency of any etiology; or (4) other serious maternal diseases such as diabetes mellitus, heart disease, Rh isoimmunization, or connective tissue disease.

During the period under study there were 29,484 deliveries at Parkland Memorial Hospital, of which 3838 (13.5%) were excluded from analysis for the reasons listed above. The remaining 24,646 deliveries were divided into four groups as follows: group 1, women pregnant for the first time who carried a pregnancy to viability (true primigravidas); group 2, women who had

an abortion (either spontaneous or induced) in the first pregnancy, but who carried the second pregnancy to viability (virtual primigravidas); group 3, women carrying two successive pregnancies to viability (true secundigravidas); and group 4, women pregnant more than twice.

The incidence of pregnancy-induced hypertension in these four groups is shown in Table I. With use of  $\chi^2$  analysis, the incidence of pregnancy-induced hypertension in group 1 was significantly higher than that in the other three groups (1 compared to 2,  $p < 0.05$ ; 1 compared to 3 and 4,  $p < 0.001$ ). Furthermore, the incidence of pregnancy-induced hypertension in group 2 was significantly greater than the incidence of pregnancy-induced hypertension in groups 3 and 4 ( $p < 0.001$ ). There was no difference in the incidence of pregnancy-induced hypertension between groups 3 and 4.

The 25.4% incidence of pregnancy-induced hypertension in our population of primigravid women (group 1) is somewhat higher than the 22.2% incidence reported by MacGillivray.<sup>3</sup> Similarly, in our patients who had the first pregnancy terminated by abortion (group 2) the incidence of pregnancy-induced hypertension was 22.3%, as compared with the incidence of 15.7% reported by MacGillivray. Our conclusion with respect to the incidence of pregnancy-induced hypertension in these two groups is, nevertheless, the same as that reached by MacGillivray, namely, that an abortion in the first pregnancy affords a degree of protection against the development of pregnancy-induced hypertension in the subsequent pregnancy. It appears, however, that the degree of protection is small compared to that afforded by a completed pregnancy. In the present study the overall incidence of pregnancy-induced hypertension in groups 3 and 4 was 9.9%, which is close to the rate of 8.0% reported by MacGillivray. The observation of similar rates of pregnancy-induced hypertension in groups 3 and 4 is suggestive that additional completed pregnancies do not provide any greater protection against pregnancy-induced hypertension than does the first pregnancy. In

summary, the results reported in the present study are consistent with MacGillivray's conclusion that an abortion in the first pregnancy confers some protection against pregnancy-induced hypertension in the subsequent pregnancy. Quantitatively, however, the degree of protection is small.

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## Male pseudohermaphroditism due to 17-ketoreductase deficiency: Report of a case without gynecomastia and without vaginal pouch

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A black adult subject, exhibiting complete virilization at puberty with psychological female orientation, was investigated. An older sister had similar physical habitus. Hormonal data showed an androstenedione/testosterone ratio  $>1$ . This led us to the diagnosis of 17-ketoreductase deficiency, with possible genetic transmission of the defect. (*AM J OBSTET GYNECOL* 1986;154:148-9.)

**Key words:** Male pseudohermaphroditism, 17-ketoreductase deficiency

Male pseudohermaphroditism due to 17-ketoreductase deficiency was first described in 1971 by Saez et al.; only a few cases have been published.<sup>1</sup> Patients are generally described as female with primary amenorrhea and/or virilization or as male with pseudohermaphroditism and gynecomastia. This paper concerns an adult patient exhibiting complete virilization (except for hypospadias) at puberty and a female psychological orientation.

#### Case report

The patient, a black subject originating from Burundi (Africa), was 29 years old when referred and investigated. This patient had been reared as female, despite mild clitoromegaly and inguinal gonads, because of a perineal hypospadias that was present at birth. The mother had received no medications during

pregnancy. At puberty, the patient had developed masculine features including facial hair, male body habitus, and growth of an erect penis. At the time of evaluation she was director of a Catholic school for girls and was complaining of socially inappropriate erections and of the impossibility of normal social, sexual, and familial life as a female. She asked for complete feminization, although she was only attracted to women. She described an older "sister" with a similar physical evolution and a brother suffering from impotence.

At physical examination, she presented as a lean male (176 cm, 52 kg) with perineal hypospadias, a penis 7 cm in length, and bilateral cryptorchidism. Neither gynecomastia nor a vaginal pouch was present. Since she was black, a possible difference in pigmentation could not be evidenced. The voice was high-pitched.

Karyotype was 46,XY. No remains of the müllerian system were demonstrated at abdominal echography or at intravenous urography. A sperm count could not be obtained because of the reluctance of the patient. Hormonal determinations are shown in Table I. Under basal conditions, luteinizing hormone and follicle-stimulating hormone were elevated, testosterone levels were low, contrasting with high values of androstenedione, and the estrone/estradiol ratio was  $>1$ . Dehydroepiandrosterone sulfate concentrations were at the upper

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**Table I.** Hormonal values

<i>Hormone</i>	<i>Mean basal value (n = 5)</i>	<i>After adrenocorticotrophic hormone</i>	<i>Before human chorionic gonadotropin</i>	<i>After human chorionic gonadotropin</i>	<i>Spermatic vein value</i>	<i>Value after castration</i>
Luteinizing hormone (mIU/ml)	39	—	—	—	—	—
Follicle-stimulating hormone (mIU/ml)	61	—	—	—	—	—
Estrone (pg/ml)	235	180	120	220	—	—
Estradiol (pg/ml)	71	41	40	34	—	—
Androstenedione (ng/ml)	5.3	3.6	3.7	9.0	31.0	1.4
Testosterone (ng/ml)	3.1	2.0	3.0	5.4	17.5	0.3
Dihydrotestosterone (ng/ml)	0.58	—	0.56	1.60	—	—
17-Hydroxyprogesterone (ng/ml)	1.51	1.75	1.55	4.05	6.5	0.45
Dehydroepiandrosterone sulfate (ng/ml)	2584	7300	—	2195	≤10	2730
Androstenedione/testosterone	1.7	1.8	1.2	1.7	1.8	—
Estrone/estradiol	3.3	4.4	3.0	6.5	—	—

For adrenocorticotrophic hormone stimulation test: 2 mg of  $\beta^{1-24}$  adrenocorticotrophic hormone per day for 3 days, intramuscularly. For human chorionic gonadotropin stimulation test: 1500 IU every 2 days for 6 days, intramuscularly. Except for the spermatic vein, all hormonal determinations were obtained from venous peripheral blood.

limit of the normal range. After administration of adrenocorticotrophic hormone, there was a sharp increase of dehydroepiandrosterone sulfate, while testosterone, androstenedione, and estrogens were decreased, with androstenedione/testosterone and estrone/estradiol ratios still  $>1$ . Human chorionic gonadotropin stimulation, performed 5 days after the adrenocorticotrophic hormone test, was followed by a trebling of androstenedione from a basal value of 3.7 ng/ml to a peak level of 9.0 ng/ml, while testosterone was only normalized. The high testosterone and androstenedione levels found in the spermatic vein (with androstenedione/testosterone  $>1$ ) confirmed the testicular origin of these androgens. The androstenedione/testosterone ratio obtained under these various conditions was rather constant, averaging 1.7 with a SD of 0.2 ( $n = 9$ ).

This led us to the diagnosis of a 17-ketoreductase deficiency. Treatment consisted of castration, removal of the penis, construction of a neovagina, and mammary prosthesis (performed by Dr. M. Lejour). Leydig cell hyperplasia and atrophy of tubules were found at histologic examination of the gonads. Only scarce germinal elements were in evidence.

### Comment

This is the first observation of 17-ketoreductase deficiency reported in a black subject. This pathologic condition is uncommon and only one familial case with late diagnosis has been previously reported.<sup>2</sup> Our observation supports the hypothesis of the genetic transmission of the defect. In contrast with previous cases,<sup>1</sup> this patient presented a male physical body habitus without gynecomastia and without a vaginal pouch. Since the patient was reared as a female and because of severe hypospadias, the only possible treatment was complete surgical feminization.

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# Coronary artery disease in diabetic pregnancies

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Severe vascular complications of diabetes mellitus include myocardial infarction and when this occurs during pregnancy it is associated with a high risk of maternal mortality. In the absence of myocardial infarction, information is unavailable on pregnancy outcome in diabetic patients with severe coronary artery disease or with prior coronary artery bypass graft. Such a case is presented together with a review of the literature. (AM J OBSTET GYNECOL 1986;154:150-1.)

**Key words:** Diabetes mellitus, coronary artery disease, pregnancy

The pregnant diabetic patient with coronary artery disease including a history of myocardial infarctions is at great risk of death.<sup>1</sup> There is no information currently available on pregnancy in diabetic patients without myocardial infarction yet with significant coronary artery disease. The purpose of this communication is (1) to report a successful gestational outcome in a patient with severe diabetic coronary artery disease as well as other forms of vascular complications (White Class HFR) and (2) to review the literature on the subject.

## Case report

A 32-year-old primigravid woman with diabetes mellitus for 17 years and known vascular complications (coronary artery disease, nephropathy, and retinopathy) experienced severe angina requiring hospitalization 2 years prior to the current pregnancy. The electrocardiogram revealed T-wave inversions in the precordial leads but without cardiac muscle enzyme changes. Cardiac catheterization done because of persistent angina refractory to medications revealed almost complete occlusion (>95%) of the left anterior descending coronary artery. A percutaneous angioplasty was unsuccessfully attempted, and there was an acute onset of chest pains, bradycardia, and hypertension. The electrocardiogram showed "ST" segment elevations. An emergency coronary artery bypass graft to the left anterior descending artery was performed. She subsequently did well and the electrocardiogram in early pregnancy was normal. Fig. 1 outlines glycemic control and hematologic-renal status throughout the preg-

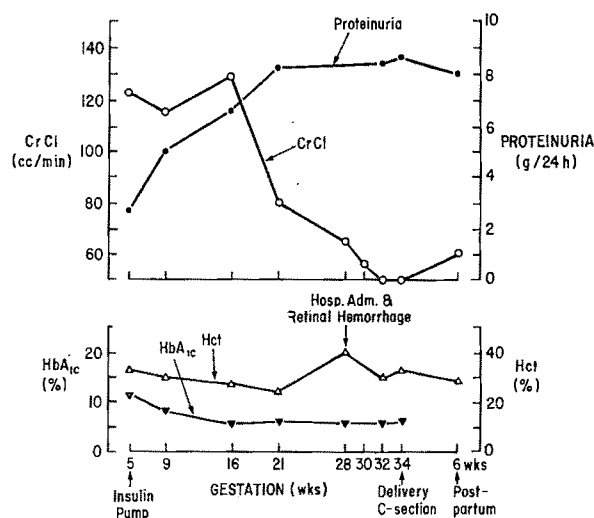


Fig. 1. Gestational profile of renal function (creatinine clearance, proteinuria), glycemic control (hemoglobin A<sub>1c</sub>), and red blood cell mass.

nancy. She did well until approximately 25 weeks' gestation when she developed the acute onset of edema in the lower extremities and sacral area, as well as hypertension (140 to 150/75 to 90 mm Hg). These conditions were successfully treated with bed rest and hydrochlorothiazide. An abrupt decrease in vision occurred secondary to vitreous hemorrhages and required laser therapy. Cardiac status remained stable without angina or electrocardiogram changes. The pregnancy was allowed to continue with intensive fetomaternal surveillance. At 34 weeks' gestation, because of continued deterioration of nephropathy and retinopathy, fetal pulmonic studies were done and revealed a lecithin/sphingomyelin ratio of 1.3 and absent phosphatidylglycerol. Following a 48-hour course of betamethasone, the patient was delivered by cesarean section of a male infant, weighing 4 pounds, 2 ounces, with Apgar scores of 7 and 8 at 1 and 2 minutes, respectively. After the operation, the renal status of the patient improved

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**Table I.** Outcome of pregnancies complicated by diabetes-associated coronary artery disease

Case No.	Author	Gravidity/parity	Age (yr)	White's classification	Type of coronary disease	Time of occurrence	Outcome	
							Maternal	Fetal
1	Brock et al. (JAMA 1953;152:1030)	4/4	34	B	MI	First trimester	Survived	Survived
2	Siegler et al. (Obstet Gynecol 1956;7:306)	1/1	38	B	MI	First trimester	Survived	Survived
3	Delaney and Ptacek (AM J OBSTET GYNECOL 1970;106:550)	—	32	B	MI	Third trimester	Died	—
4	White (J Am Med Women's Assoc 1972;27:293)	—	35+	B	MI	First trimester	Died	Aborted
5		—	35+	B	MI	First trimester	Died	Aborted
6		—	35+	B	MI	First trimester	Died	Aborted
7	Hubbard (Clin Obstet Gynecol 1975;18:27)	1/1	36	R	MI	First trimester	Died	Aborted
8	Hare and White (Diabetes 1977;26:953)	—	—	—	MI	Prior to pregnancy	Survived*	Survived
9		—	—	—	MI	Third trimester	Died	Survived
10		—	—	—	MI	4 wk postpartum	Died	Survived
11		—	—	—	MI	4 wk postpartum	Died	Died
12	Silfen (Obstet Gynecol 1980;55:749)	1/1	23	D	MI	Prior to pregnancy	Survived	Survived
13	Reece et al. (current case)	1/1	32	HFR	Severe angina, occlusion of LAD artery	Prior to pregnancy	Survived*	Survived

— = Information not reported. LAD = Left anterior descending. MI = Myocardial infarction.

\*Coronary artery bypass procedure prior to pregnancy.

steadily, the retinopathy was responsive to laser therapy, and the cardiac condition remained stable. The neonate, although experiencing moderate respiratory distress, did well and was discharged from the hospital in 3 weeks.

#### Comment

Prior to the discovery of insulin there was a high incidence of maternal mortality associated with diabetic complications of infection and ketoacidosis. Today, with the availability and utilization of insulin, a new dimension of vascular complications confronts the clinician: retinopathy, nephropathy, and cardiopathy. Coronary heart disease is rarely encountered in pregnant diabetic patients. Table I outlines the outcome of the 12 reported cases of diabetes-associated coronary artery disease in pregnancy.

Comparative studies of diabetics and nondiabetics reveal a significantly greater degree of diffuse coronary artery disease, increased numbers of coronary artery bypass grafts, higher morbidity and mortality, and a lower long-term survival rate among diabetic patients.<sup>2</sup>

In addition, coronary artery bypass grafting has qualitatively and quantitatively been associated with improved survival; however, the effect of pregnancy in these patients has not been studied. Interestingly, the only two cases in the literature (including the current report) in which coronary artery bypass grafting was performed resulted in successful maternal and neonatal outcomes. Although it is tempting to suggest that pregnancy outcome seems to be improved by coronary artery bypass grafting prior to pregnancy, data in support of this statement are currently unavailable.

The case presented demonstrates that despite severe vasculopathy, including coronary artery disease, meticulous metabolic control, appropriate maternal and fetal surveillance, and a timely delivery can produce a successful fetal and maternal outcome.

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# Multiple peripheral pulmonic stenosis in pregnancy

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Multiple peripheral pulmonic stenosis is a rare congenital cardiac disorder. The clinical course of this disease in pregnancy is not established. We report the benign obstetric course of a patient whose pregnancy was complicated by peripheral pulmonic stenosis and pulmonary hypertension. (AM J OBSTET GYNECOL 1986;154:152-3.)

**Key words:** Peripheral pulmonic stenosis, cardiac disease in pregnancy

Although pulmonary stenosis is usually associated with a benign course during gestation, pulmonary hypertension often leads to cardiac decompensation and maternal mortality. This case report concerns pregnancy in a patient with multiple congenital peripheral pulmonic stenosis and pulmonary hypertension managed with aggressive antepartum monitoring of cardiac function. This is the second case of this disorder complicating pregnancy described in world literature.

## Case report

C. C. is a 24-year-old black woman, gravida 1, para 0, who registered at George Washington University Medical Center for prenatal care at 12 weeks' gestation. She was the product of a normal pregnancy and delivery, without known maternal exposure to rubella. There is no familial history of cardiac disease. At 2 years of age a cardiac murmur was first heard. Her parents were told that she had an outflow obstruction on the left side of the heart but that she could be treated medically. Her growth and development were described as slow. At 9 years of age, she complained of difficulty climbing stairs and was unable to participate in physical education classes. She was referred to the National Institutes of Health for evaluation. Cardiac examination revealed a prominent right ventricular impulse, normal S1, split S2 with striking prominence of the pulmonic closing component, and a grade III/VI blowing systolic ejection murmur over the entire chest anteriorly and posteriorly with a short diastolic component in the infraclavicular areas. The lungs were clear and peripheral pulses were normal and equal. Electrocardiography showed right ventricular hypertrophy with right axis deviation. Echocardiogram and chest x-ray studies were within normal limits. Cardiac catheterization showed peak systolic pulmonary artery and right ventricle pressures equaling 80 mm Hg. A pulmonary arteriogram revealed distal multiple pulmonary stenoses

of type III with no discrete obstruction of the main pulmonary artery branches. The patient was told that no medical or surgical therapy was indicated. At age 16 she complained of palpitations and was treated with digoxin, which she discontinued because of lack of improvement.

She was without complaints at the time of registration for prenatal care. The physical examination was unremarkable except for the grade III/VI systolic ejection murmur, which was unchanged since childhood. Cardiac catheterization showed right axial mean pressure of 10 mm Hg, right ventricle pressure of 70/16 mm Hg, pulmonary artery pressure of 65/18 mm Hg, pulmonary capillary wedge pressure of 10 to 12 mm Hg, radial artery pressure of 130/70 mm Hg, and radial artery oxygen saturation of 96% with oxygen saturation of 78% for right atrium, ventricle, and pulmonary artery.

The case was followed in the high-risk obstetric clinic with cardiology consultation. The antepartum course was benign with good fundal growth. Blood pressure remained between 110 to 128/60 to 68 throughout the pregnancy. Repeat catheterization at 26 weeks showed a decrease of pulmonary artery pressures to 55/10 mm Hg with pulmonary capillary wedge pressure of 8 mm Hg. Pulmonary function tests showed a mild restrictive ventilatory defect with mild airflow obstruction. Except for an *Escherichia coli* urinary tract infection treated with ampicillin she continued to do well throughout pregnancy. There was mild restriction of activities during the third trimester, prompted largely by the patient's dyspnea associated with exertion.

At 39 weeks she was admitted for induction of labor with cardiac monitoring with use of a Swan-Ganz catheter. Physical examination showed pulse of 86 bpm, blood pressure of 120/70 mm Hg, neck veins were moderately distended, and the cardiac murmur was unchanged. Fundal height measured 38 cm with the fetus in vertex presentation, fetal heart tones were 140/min, estimated fetal weight was 6 pounds. The cervix was dilated 2 cm, 75% effaced with the vertex at 0 station. Cardiac pressures before induction were as follows: right ventricle, 60/5 mm Hg; pulmonary artery, 55/2 mm Hg; mean pulmonary artery, 24 mm Hg; pulmonary capillary wedge pressure, 3 to 6 mm Hg. Induction was begun with Pitocin, and artificial rupture of the

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membranes yielded clear fluid. The mean pulmonary artery pressure ranged between 24 to 31 mm Hg, peak pulmonary artery pressure was 53 to 65 mm Hg, and end diastolic pressure was -7 to +8 mm Hg. After an 8-hour labor, a low-forceps delivery over a midline episiotomy with pudendal block resulted in delivery of a male infant with Apgar scores of 8 and 9 at 1 and 5 minutes and weighing 6 pounds, 3 ounces. At the time of delivery systemic blood pressure was 139/75, pulmonary artery pressure was 69/-9 mm Hg, with mean pressure of 23 mm Hg. Two hours post partum, pressures rose to a maximum pulmonary artery pressure of 79/11 mm Hg and mean pressure of 35, but gradually decreased with spontaneous diuresis. The Swan-Ganz catheter was removed 14 hours after delivery, at which time blood pressure was 130/72 mm Hg, pulse was 72/min, pulmonary artery pressure was 65/8 mm Hg, right ventricle pressure was 66/6 mm Hg, and pulmonary capillary wedge pressure was 6 to 8 mm Hg. Intravenous fluids were carefully controlled with a 24-hour input of 2850 ml and output of 2650 ml. The mother and infant both remained well and were discharged on the fourth day after delivery.

#### Comment

Multiple peripheral pulmonic stenosis is a rare disease accounting for 2% to 4% of the total number of congenital cardiac abnormalities. The anatomic malformation consists of multiple coarctations in the distal branches of the pulmonary arteries. Elevation of main pulmonary artery pressure indicates the severity of pul-

monary hypertension; however, with normal pulmonary capillary wedge pressures the functional course of the disease is usually benign. There has been virtually no experience with the course of this disease during pregnancy.

Togo et al.<sup>1</sup> reported the only other case of multiple peripheral pulmonic stenosis complicating pregnancy in a Japanese patient. The patient's course was similar to the case presented here including a postpartum elevation of pressures without cardiopulmonary decompensation.

The ability to monitor cardiac hemodynamics with the use of the Swan-Ganz catheter allows the obstetrician to manage critically ill patients in whom pregnancy and delivery threaten maternal survival.<sup>2</sup> This case supports the belief that women with uncomplicated multiple peripheral pulmonic stenosis can probably expect to have a benign perinatal course despite pulmonary hypertension. Nevertheless, until further experience is gained, we recommend continued careful cardiac monitoring of these patients to further our understanding of this disorder's relationship to pregnancy and to optimize obstetric outcome.

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## Neonatal alloimmune thrombocytopenic purpura: A case report

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We report a case of a sibling pair with neonatal alloimmune thrombocytopenic purpura. Serial antepartum platelet alloantibody quantitation by an enzyme-linked immunoabsorbent assay revealed rising antibody titers during advancing gestation. We discuss the implications of this finding in the antepartum diagnosis of neonatal alloimmune thrombocytopenic purpura, a rare, but frequently fatal disorder. (*AM J OBSTET GYNECOL* 1986;154:153-5.)

**Key words:** Alloimmune thrombocytopenic purpura, platelet antigen incompatibility

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Maternal-fetal platelet antigen incompatibility is common. Rarely, incompatibility of certain platelet-specific antigens, for instance, PLA1, DUZO, and Bak<sup>a</sup>, and of some platelet human lymphocyte antigens, for instance, HLA-2, results in maternal sensitization, placental transfer of IgG, and fetal platelet binding that manifests at birth as neonatal alloimmune thrombocytopenic purpura. The mortality rate due to central nervous system sequelae is 12% in the absence of treat-

ment, Reliable antepartum diagnosis is currently unavailable. We report the first case in which an enzyme-linked immunoabsorbent assay was used for serial antepartum quantitation of maternal indirect platelet alloantibody.

### Case report

A 27-year-old, para 0, white woman, after an uncomplicated pregnancy, had a normal spontaneous delivery at term, while under epidural anesthesia, of a 7 pound female infant with Apgars of 9 and 10 at 1 and 5 minutes, respectively. The infant's examination was remarkable for extensive petechiae with a platelet count of 17,000/ $\mu$ l shortly following birth. Blood cultures and TORCH (toxoplasmosis, rubella virus, cytomegalovirus, herpesvirus) titers were negative. A bone marrow aspirate revealed a normal number and normal morphologic features of megakaryocytes. The maternal platelet count was normal and the maternal direct and indirect Coombs tests were negative. Except for a bilirubin level of 15 mg/dl, the infant did well without specific therapy of neonatal thrombocytopenia. At 2 months of age platelet count was 212,000/ $\mu$ l and at 6 years of age she was normally developed. Postpartum platelet typing revealed the mother to be PLA1 negative, PLE1 and HLA-2 positive; the father was PLA1 positive. An immunofluorescent indirect antihuman globulin test identified an antiplatelet alloantibody in the mother's sera, which was felt to be the cause of the neonate's thrombocytopenia. The same mother was seen for the initial visit during her second pregnancy at 7 weeks' gestation, by dates and size and ultrasound determination. The pregnant mother's physical examination was unremarkable. At that time the maternal platelet count was 245,000/ $\mu$ l. At 22 weeks of this gestation an indirect enzyme-linked immunoabsorbent assay first detected maternal platelet antibody. By 32 weeks the platelet antibody was positive in all dilutions of the maternal serum. At this time sonography confirmed the gestational age by dates and size. Weekly nonstress tests after 32 gestational weeks were reactive. Ultrasonography at 34 weeks revealed no evidence of fetal intracranial hemorrhage or ventricular dilatation. The lecithin/sphingomyelin ratio and presence of phosphatidylglycerol were consistent with fetal pulmonary maturity. The infant was felt to be at risk of severe neonatal alloimmune thrombocytopenic purpura because of the sibling's birth history and the serologic platelet testing. Therefore, after consultation with the neonatologist, we proceeded with an elective lower uterine segment transverse cesarean section at the thirty-fourth gestational week with the patient under epidural anesthesia. The patient was delivered atraumatically of a 2590 gm female with Apgar scores of 7 and 8 at 1 and 5 minutes and a Dubowitz gestational age score of 34 weeks. The cord blood platelet count was 26,000/ $\mu$ l. At 1 hour following birth, scattered petechiae and ecchymoses were noted and the platelet count was 17,000/ $\mu$ l with a simultaneous hematocrit of

43%. In keeping with our nursery policies, two units of AB plasma-washed maternal platelets, obtained by platelet pheresis on the day preceding delivery, were transfused into the neonate, resulting in a sustained posttransfusion platelet count of 47,000 to 65,000 by day 2 of life. Except for mild jaundice this infant did not experience adverse sequelae. At 2 months of age the infant had a normal platelet count and at 1 year of age was normally developed. The mother had a benign postpartum course.

### Comment

Because a routine serologic diagnostic assay is unavailable and because of the infrequent occurrence of neonatal alloimmune thrombocytopenic purpura in infants born of primigravid women, this disorder is usually diagnosed only after birth. Neonatal alloimmune thrombocytopenic purpura is suspected when transfusion of maternal platelets, which lack the offending antigen, produce a prompt sustained improvement of the neonatal platelet count following the unsuccessful transfusion of random donor platelets. The finding of a platelet-specific antigen shared by the infant and father, which is absent from the mother's platelets, and the identification of a maternal indirect platelet alloantibody may substantiate the diagnosis. The disorder frequently recurs during subsequent pregnancies.

Antibodies against platelet-specific antigens are only rarely complement fixing. Less frequently associated with neonatal alloimmune thrombocytopenic purpura are platelet alloantibodies with human lymphocyte specificity, which are complement fixing but found in 1% to 3% of normal pregnancies. Only recently have reproducible, sensitive assays of antibody against platelet-specific antigens been available, but they also detect platelet human lymphocyte antibodies and they detect antibody remote from the immunizing pregnancy. A qualitative or single quantitative determination by such assays, which rely on antihuman globulin linked to fluorescein, radioactive iodine, or sheep red blood cells, would appear to lack specificity for the antepartum diagnosis of neonatal alloimmune thrombocytopenic purpura. The enzyme-linked immunoabsorbent assay is a sensitive test of platelet antibody in a variety of disorders. However, there is limited experience with the enzyme-linked immunoabsorbent assay for platelet alloantibody detection. There are only two cases of neonatal alloimmune thrombocytopenic purpura in which an enzyme-linked immunoabsorbent assay detected antibody in the maternal postpartum sera.<sup>1</sup> In the only previous report in which a sensitive assay was used for antepartum serial quantitation of maternal indirect platelet alloantibody in a case of neonatal alloimmune thrombocytopenic purpura, the sheep red blood cell antihuman IgG assay detected rising titers with ad-



vancing gestation.<sup>2</sup> Using an enzyme-linked immunoabsorbent assay, we noted a similar relationship of the antibody titer to advancing gestation. Whether this is a specific finding of neonatal alloimmune thrombocytopenic purpura affecting a current gestation will require further study. Antepartum recognition of the disorder would aid in planning timely management. Antepartum maternal platelet pheresis to make available compatible platelets for prompt transfusion of the neonate, antepartum administration of corticosteroids, and cesarean section delivery might prevent or ameliorate central nervous system sequelae.

We thank Michelle C. Suthantheran for her excellent technical assistance.

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## Ovarian pregnancy with a contralateral corpus luteum: Case report

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A case is described of a woman who was found to have an ovarian pregnancy on the left with a corpus luteum on the right ovary. This was determined by both pelvic sonography and laparotomy. The case did fulfill the four criteria of Spiegelberg. (*Am J Obstet Gynecol* 1986;154:155-6.)

**Key words:** Ovarian pregnancy, corpus luteum

Ovarian pregnancy is a rare form of ectopic gestation with approximately 300 cases reported in the literature.<sup>1</sup> The etiology remains obscure. Ectopic tubal pregnancies with a contralateral corpus luteum have been previously reported.<sup>2</sup> Reported is a case of an ovarian pregnancy with a contralateral corpus luteum, which we believe is the first one reported.

The symptoms that prompted the diagnostic laparoscopy and the subsequent laparotomy proved to be secondary to a leaking corpus luteum cyst of pregnancy. Thus we were able to have a clear view of the ovaries and tubes by sonography and by observation at the time of operation without the distorted image one gets following a ruptured ectopic pregnancy.

#### Case report

The patient, a 24-year-old woman, had a 16-month history of infertility. She had a seemingly normal menses and was asked to come in on the twelfth day of the

menstrual cycle for a pelvic sonogram to evaluate follicular maturation. She was found to have a follicle on the right ovary that measured 18.3 mm. No follicles or cysts >10 mm were seen on the left. When she returned in 2 days for a check on the release of the egg, the cyst had grown to 24 mm. By day 18 the cyst had grown to 34.7 mm and the patient was thought to have a luteinized unruptured follicle syndrome. However, because of severe right lower quadrant abdominal pain, a test for the  $\beta$ -subunit of human chorionic gonadotropin was performed. The level was 7522 mIU/ml, which led to the laparoscopy and subsequent laparotomy. The presumptive preoperative diagnosis was an ectopic pregnancy, probably in the right tube.

At laparoscopy there was approximately 100 to 150 ml of dark blood and clots. A large cyst of approximately 3 to 4 cm in diameter was seen on the right ovary. Blood was oozing from it and it appeared to be a corpus luteum cyst. Careful inspection of the left ovary revealed an ovarian pregnancy at the distal pole, with confirmation at laparotomy. There were products of conception on the surface of the left ovary that were adherent to the posterior leaf of the broad ligament and the posterior uterine serosa via the ovarian ligament. The left fallopian tube appeared to be free and uninvolved. It was of normal length with a well-developed fimbrial portion. There were no endometrial im-

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plants seen or any adhesions elsewhere in the pelvis. The right tube appeared perfectly normal.

At laparotomy the left tube was identified with tubal forceps and examined in its entirety. No pregnancy could be seen within the tube and the fimbriated end appeared normal and uninvolved. The pregnancy was excised from the distal pole of the left ovary along with a part of the normal ovary.

The pathology report confirmed the presence of an ectopic pregnancy along with normal ovarian tissue.

#### Comment

A case of an ovarian pregnancy with a contralateral corpus luteum is described. We are not aware of another such case although a contralateral corpus luteum in tubal pregnancies has been described previously.<sup>2</sup> This case did fulfill the four criteria to establish an ovarian pregnancy as set forth by Spiegelberg in 1878: (1) The tube on the affected side was normal; (2) the

gestational sac occupied the normal position of the ovary (distal pole); (3) the sac was connected to the uterus by the ovarian ligament; (4) unquestionable ovarian tissue was demonstrated in the wall of the sac.

This case thus supports the hypothesis that an ovarian pregnancy may arise outside the ovary followed by either intrafollicular or extrafollicular implantation. Possible explanations for this ovarian pregnancy would include: (1) the menstrual reflux theory of Iffy with the zygote transversing the fallopian tube to the distal pole of the left ovary, (2) intraperitoneal fertilization, or (3) superovulation.

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## Constrictive pericarditis and pregnancy

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A case discussing the medical management of a 30-year-old gravid patient with recurrent pericarditis and pericardial constriction secondary to juvenile rheumatoid arthritis is presented. (*AM J OBSTET GYNECOL* 1986;154:156-7.)

**Key words:** Constrictive pericarditis, pregnancy, labor

There have been few case reports of constrictive pericarditis in pregnancy. This report presents the medical management of a 30-year-old gravid patient with recurrent pericarditis and pericardial constriction secondary to juvenile rheumatoid arthritis.

#### Case report

A 30-year-old white woman, gravida 2, para 1, was admitted at 38 weeks' gestation for Pitocin induction of labor. She had a 12-year history of recurrent pericarditis secondary to juvenile rheumatoid arthritis. Since then she had experienced episodes of acute peri-

carditis manifested as pleuritic chest pain, dyspnea, and atrial tachyarrhythmias, which had been treated with tapering courses of prednisone and 975 mg of aspirin three times daily. Her first child had been delivered vaginally 5 years previously with no complications during a period of remission. She discontinued aspirin therapy before pregnancy. However, in the second trimester she had a relapse but improved with bed rest. Treatment with steroids was refused. At the beginning of the third trimester she again experienced a relapse and was referred for consultation.

At 32 weeks' gestation physical examination revealed a pregnant woman who appeared uncomfortable and dyspneic at rest. Blood pressure while sitting was 120/90 mm Hg, heart rate was 135 bpm and regular, respiratory rate was 28/min. There was jugular venous distention of 9 cm of water with a prominent diastolic Y descent. Cardiac examination revealed a pericardial S3 "knock" without murmurs or displaced apical impulse. The uterus was enlarged, compatible with estimated gestational age. Mild ankle and finger edema was present. The rest of the physical examination was

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normal. The electrocardiogram showed sinus tachycardia with nonspecific ST-T wave abnormalities and borderline low voltage. An echocardiogram showed a concentrically thickened pericardium and small pericardial effusion with normal chamber size and appearance of the cardiac valves.

The patient was managed with strict bed rest and experienced improvement in symptoms. Because chest pain was not an active complaint, steroids were not given. However, the symptoms began to recur, and it was decided to induce labor at 38 weeks' gestation.

On the morning of induction, blood pressure was 108/70 mm Hg, heart rate was 120 bpm, respiratory rate was 18/min, and there was no jugular venous distention. A Swan-Ganz catheter was inserted. The initial intracardiac pressures were within normal limits, showing a mean right atrial pressure of 3 mm Hg, right ventricular pressure of 21/3 mm Hg, and mean pulmonary capillary wedge pressure of 8 mm Hg. After moderate intravenous volume expansion, epidural anesthesia was successfully induced with 12 ml of 0.25% Marcaine, producing a sensory block at the level of T-8. The patient received an average of 8 ml/hr of 0.25% Marcaine throughout induction, increasing the sensory block to T-10. As the rate of uterine contractions progressed, the concentration of Marcaine was increased to 0.5%, producing a sensory and partial motor block to level T-10.

During labor the right atrial pressure rose to an elevated level of 12 mm Hg, associated with the appearance of an abnormal right atrial waveform with a prominent and steep diastolic Y descent and a right ventricular waveform with a "dip-plateau" configuration. Cardiac output was 7 L/min during labor and rose to 12 L/min in the active phase. A 6 pound, 9 ounce healthy infant was delivered by low forceps without complications after 7 hours of labor. Mother and child were discharged on the fifth postpartum day.

Two weeks after delivery the patient felt well. However, 3 months after delivery she experienced recurrent episodic mild pleuritic pain and palpitations exacerbated by exercise. Pericardiectomy is under consideration before she begins a third desired pregnancy.

### Comment

This is a unique case of chronic constrictive pericarditis caused by juvenile rheumatoid arthritis in

pregnancy. However, there have been few case reports of constrictive pericarditis in pregnancy.<sup>1</sup> Constrictive pericarditis is a disorder in which a chronically thickened and fibrotic pericardium limits cardiac filling. It is a serious threat to both the pregnant woman and the fetus. Cardiac output increases by as much as 40% to 50% in pregnancy and by 50% during active labor. An increase in either stroke volume or heart rate can produce the augmented cardiac output. Patients with severe constrictive pericarditis usually have a limited stroke volume caused by poor diastolic filling. They compensate for this with an increase in their heart rate to meet the rapidly increasing demands.

This case illustrates the principles of management of the pregnant patient with constrictive physiology. In most patients, management can be conservative. Restriction of activity is useful in reducing cardiac demand. Diuresis should be avoided, since it may limit right ventricular filling. Sinus tachycardia is a crucial compensatory mechanism to sustain cardiac output when the heart's ability to augment stroke volume is limited, and it should not be suppressed pharmacologically. Digoxin or  $\beta$ -adrenergic blockers are only indicated for control of atrial fibrillation or flutter. Steroids do not change pericardial compliance and have no role unless there is evidence of active inflammation. Swan-Ganz floatation catheterization is extremely useful in monitoring intracardiac pressures and cardiac output to avoid excessive and disastrous drops in right heart filling pressure during active labor and to forewarn against excessive iatrogenic volume expansion.

To minimize the pain and anxiety associated with labor, epidural anesthesia was used with careful attention to prevent vasodilation. In patients with more severe constrictive pericarditis, pericardiectomy has been successfully performed during pregnancy.<sup>2</sup>

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# Serum C-reactive protein determination in acute pelvic inflammatory disease

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We have studied the role of serum C-reactive protein determination in the diagnosis of acute pelvic inflammatory disease. Acute-phase serum C-reactive protein concentration reflected the extent and the severity of pelvic inflammatory disease more closely than erythrocyte sedimentation rate or white blood cell count determinations. We recommend that both C-reactive protein concentration and erythrocyte sedimentation rate should be routinely used to augment the clinical diagnosis of pelvic inflammatory disease. (AM J OBSTET GYNECOL 1986;154:158-9.)

**Key words:** C-reactive protein, endometritis, salpingitis, pelvic inflammatory disease

Acute pelvic inflammatory disease in women is a polymicrobial infection with a wide range of symptoms and signs. Clinical criteria for the diagnosis of pelvic inflammatory disease, such as the presence of low abdominal pain, bilateral adnexal tenderness, fever, and increased erythrocyte sedimentation rate, have poor sensitivity and specificity as demonstrated by laparoscopic studies. Proper management of such patients, however, requires a simple and rapid test with relatively high predictive value. Determination of serum C-reactive protein levels has improved the rapid diagnosis of many acute bacterial infections, but only a few studies have evaluated C-reactive protein levels in women with pelvic inflammatory disease<sup>1</sup> or in women with laparoscopically diagnosed salpingitis.<sup>2</sup>

## Material and methods

We studied by laparoscopy and endometrial sampling 41 consecutive women with suspected acute pelvic inflammatory disease at the Department of Obstetrics and Gynecology, University of Tampere, Finland. Cultures for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* were taken from the endocervix, endometrium, fallopian tubes, posterior cul-de-sac and rectum and were analyzed as described.<sup>3</sup> The histologic diagnosis of endometritis was based on the presence of plasma cells in an endometrial biopsy.<sup>3</sup> The laparoscopic criteria for acute salpingitis were as described.<sup>3</sup> Serum C-reactive

protein concentrations were measured by a turbidimetric method.<sup>4</sup>

## Results

C-reactive protein levels were significantly ( $p < 0.001$ ) higher among 26 patients with endometritis and/or salpingitis (58 mg/L, range of 0 to 170 mg/L) than among nine patients who had neither salpingitis nor endometritis (15 mg/L, range of 0 to 45 mg/L) (Table I). Women with only endometritis tended to have higher C-reactive protein concentrations than women with neither endometritis nor salpingitis, though the difference was not significant. In patients with suspected pelvic inflammatory disease the sensitivity and specificity of C-reactive protein determinations (cut-off level, 20 mg/L) in the diagnosis of pelvic inflammatory disease was 74% and 67%, respectively. Corresponding figures for erythrocyte sedimentation rate (cut-off level, 15 mm/hr) were 81% and 57%. Thus no major differences between the two tests were found. When the tests were combined so that a positive value in both tests was regarded as a true positive finding, the sensitivity was 65% and the specificity was 75%. If either one of the tests was considered to be indicative of acute salpingitis, the sensitivity of the combined tests was 91%, the specificity being 50%. C-reactive protein concentration had a positive correlation with the severity of the laparoscopically diagnosed salpingitis. However, C-reactive protein determination did not discriminate between those who had either *C. trachomatis* ( $68 \pm 39$  mg/L), *N. gonorrhoeae* ( $64 \pm 35$  mg/L), or neither ( $58 \pm 66$  mg/L) isolated from any genital tract site. Highest C-reactive protein levels were seen in two patients who had *Haemophilus influenzae* (170 mg/L) and *Escherichia coli* (155 mg/L), respectively, isolated from the fallopian tubes.

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**Table 1.** Correlation of serum C-reactive protein, erythrocyte sedimentation rate, and white blood cell count levels to the presence and severity of disease among 41 patients studied by laparoscopy and endometrial biopsy for suspected pelvic inflammatory disease

	C-reactive protein (mg/L, mean $\pm$ SD)	Erythrocyte sedimentation rate (mm/hr, mean $\pm$ SD)	White blood cell count ( $n \times 10^6$ cells, mean $\pm$ SD)
Salpingitis* (n = 26)	58 $\pm$ 49†	36 $\pm$ 22†	9.0 $\pm$ 2.7
Mild (n = 12)	39 $\pm$ 43‡	26 $\pm$ 15‡	8.8 $\pm$ 3.3
Moderate (n = 8)	69 $\pm$ 48§	35 $\pm$ 27†	9.2 $\pm$ 3.1
Severe (n = 6)	93 $\pm$ 50§	59 $\pm$ 10§	10.6 $\pm$ 2.6
Endometritis, no salpingitis (n = 6)	33 $\pm$ 46	12 $\pm$ 10	9.1 $\pm$ 3.5
No endometritis, no salpingitis (n = 9)	15 $\pm$ 10	16 $\pm$ 16	8.2 $\pm$ 2.8

\*Severity of salpingitis was assessed with use of the laparoscopic criteria of mild, moderate, and severe salpingitis.

†Difference between patients with and without salpingitis (Mann-Whitney *U* test):  $p < 0.025$ .

‡Difference between patients with and without salpingitis (Mann-Whitney *U* test):  $p < 0.05$ .

§Difference between patients with and without salpingitis (Mann-Whitney *U* test):  $p < 0.001$ .

### Comment

Our findings indicate that C-reactive protein determination is as discriminatory as erythrocyte sedimentation rate in the diagnosis of acute pelvic inflammatory disease. C-reactive protein levels seem to react already in endometritis, and rapidly increase with more severe pelvic inflammatory disease. Thus a combination of erythrocyte sedimentation rate and C-reactive protein determination might be more accurate in predicting tubal disease. Further studies should determine whether the C-reactive protein concentration is useful in monitoring the duration of antimicrobial therapy for pelvic inflammatory disease.

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## Sinusoidal fetal heart rate pattern after administration of nalbuphine hydrochloride: A case report

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Nalbuphine hydrochloride (Nubain) is a synthetic analgesic available for use during labor. It is known to possibly cause respiratory depression in the neonate, but to date there are no published reports of any intrapartum alterations of fetal heart rate. A case presentation is given of a persistent sinusoidal pattern appearing after Nubain administration. (*AM J OBSTET GYNECOL* 1986;154:159-60.)

**Key words:** Sinusoidal heart rhythm, Nubain

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A sinusoidal fetal heart rate pattern has been described as being anything from a normal variant to a sign of impending fetal death. It is most immediately associated as a pattern indicative of high-output cardiac failure, usually attributable to severe isoimmunization or hemorrhage. Occasionally, a sinusoidal pattern ap-

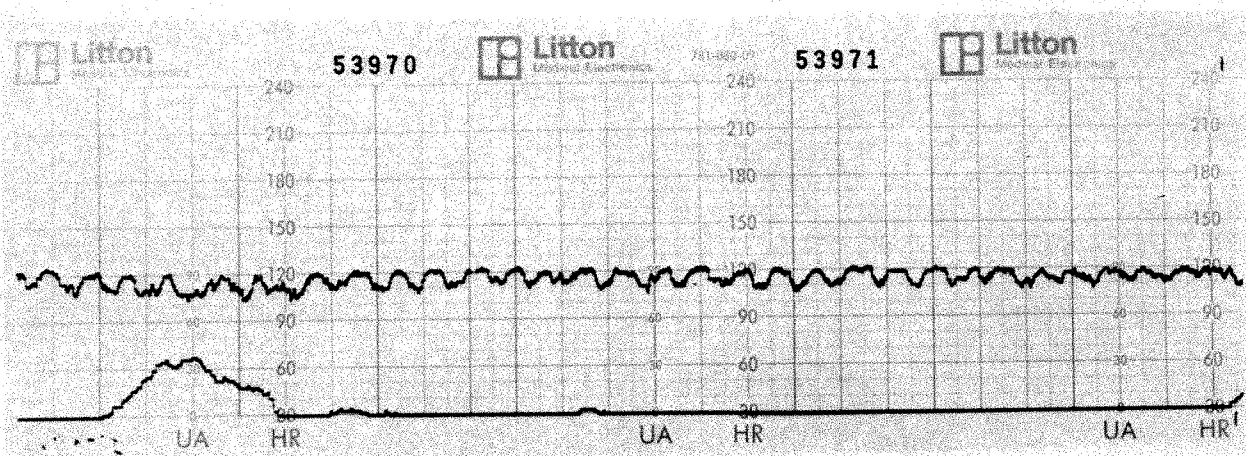


Fig. 1. Representative section of transmitted fetal heart rate tracing.

pears after the administration of a maternal analgesic, the most commonly reported being alphaprodine (Nisentil). The following case is the first report of a nalbuphine hydrochloride (Nubain)-induced sinusoidal pattern.

#### Case report

Through our FACTS\* line at The University of Connecticut Health Center, we received a tracing from a local community hospital with the following information: M. T., a 21-year-old, white woman, gravida 2, para 0, at 42 weeks' gestation by dates, had spontaneous rupture of the membranes at 9:00 AM. Amniotic fluid was originally described as "yellow tinged" and appeared then (1:30 PM at time of transmission) as "fresher" meconium. We were informed that at 10:45 AM she was given 100 mg of Vistaril intramuscularly with no change in an otherwise "reactive and normal" tracing. At 12:15 PM, 1 hour and 15 minutes before the transmitted tracing, 10 mg of Nubain was given intravenously. A definite abrupt change on the internal scalp electrode tracing reportedly occurred several minutes later, with a disturbing, sinusoidal appearance (Fig. 1). The tracing prior to Nubain administration was reported as "reactive and normal." The sinusoidal pattern, by virtue of proximity of onset after analgesia, was felt to be drug-related. The transmission contin-

ued, and despite 2 hours and 15 minutes that had elapsed since the Nubain administration, the basic pattern was unchanged. Periodic late decelerations were becoming evident, and with their presence, a persistent sinusoidal pattern, and the patient still in latent phase labor, cesarean section was performed. An "active and pink" 10 pound, 3 ounce baby girl, with Apgar scores of 8 and 9 at 1 and 5 minutes, respectively, was delivered. Both mother and baby had an uncomplicated postpartum course.

#### Comment

Nalbuphine hydrochloride is a synthetic narcotic agonist-antagonist analgesic of the phenanthrene series. It is related to both naloxone, a widely used narcotic antagonist, and oxymorphone, a potent narcotic analgesic. Its onset of action is rapid, only 2 to 3 minutes following an intravenous dose, and it is stated to be as potent an analgesic as morphine on a milligram basis. It should be specifically noted that the plasma half-life of nalbuphine is 5 hours; therefore, this may account for the longer than anticipated sinusoidal pattern. To our knowledge, this is the first reported association of nalbuphine hydrochloride and a sinusoidal fetal heart rate pattern.

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\*Fetal Assessment Consultative Transmission System.



# Less withdrawal bleeding: another reason for OGEN<sup>®</sup> (estropipate)



In a 3-month comparative trial<sup>1</sup>, OGEN and a progestogen\* induced less withdrawal bleeding than did conjugated estrogens and a progestogen.\*

It's just one more reason why OGEN is so easy to start with, and so easy to stay with.

**Percentage of women without withdrawal bleeding, taking either OGEN 1.25 or conjugated estrogens 0.625 mg<sup>1</sup>**

OGEN <sup>®</sup> 1.25	42% (19/45)
conjugated estrogens 0.625	18% (9/50)
<i>p</i> < 0.05.      Adapted from Wren and Routledge, 1982. <sup>1</sup>	

\*Both regimens were given 24 days out of 30, with levonorgestrel 0.03 mg, a progestogen, added from the 15th to 24th day.

## OGEN<sup>®</sup> (estropipate)

*Only estrone. Identical to the body's own.*



*Scored tablets make  
precise dosing easy*



**OGEN .625**

(estropipate 0.75 mg, calculated as  
sodium estrone sulfate 0.625 mg)



**OGEN 1.25**

(estropipate 1.5 mg, calculated as  
sodium estrone sulfate 1.25 mg)



**OGEN 2.5**

(estropipate 3 mg, calculated as  
sodium estrone sulfate 2.5 mg)



**OGEN 5**

(estropipate 6 mg, calculated as  
sodium estrone sulfate 5 mg)

■ OGEN tablets are scored so you can adjust dosage without writing a new prescription. It's another reason more physicians are prescribing OGEN.

See adjacent page for brief summary of prescribing information.

**Reference:**

1. Wren BG, Brown LB, Routledge DA: Differential clinical response to oestrogens after menopause. *Med J Aust* 2:329-332, 1982.

**OGEN<sup>®</sup>**  
(estropipate)

*Only estrone. Identical to the body's own.*





# **OGEN®** ESTROPIRATE TABLETS, USP Tablets

## **WARNING:** **1. ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA.**

Three independent case control studies have shown an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for prolonged periods. This risk was independent of the other known risk factors for endometrial cancer. These studies are further supported by the finding that incidence rates of endometrial cancer have increased sharply since 1969 in eight different areas of the United States with population-based cancer reporting systems, an increase which may be related to the rapidly expanding use of estrogens during the last decade.

The three case control studies reported that the risk of endometrial cancer in estrogen users was about 4.5 to 13.9 times greater than in nonusers. The risk appears to depend on both duration of treatment and on estrogen dose. In view of these findings, when estrogens are used for the treatment of menopausal symptoms, the lowest dose that will control symptoms should be utilized and medication should be discontinued as soon as possible. When prolonged treatment is medically indicated, the patient should be reassessed on at least a semiannual basis to determine the need for continued therapy. Although the evidence must be considered preliminary, one study suggests that cyclic administration of low doses of estrogen may carry less risk than continuous administration; if therefore appears prudent to utilize such a regimen.

Close clinical surveillance of all women taking estrogens is important. In all cases of undiagnosed persistent or recurring abnormal vaginal bleeding, adequate diagnostic measures should be undertaken to rule out malignancy.

There is no evidence at present that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.

## **2. OGEN SHOULD NOT BE USED DURING PREGNANCY.**

According to some investigators, the use of female sex hormones, both estrogens and progestogens, during early pregnancy may seriously damage the offspring. Studies have reported that females exposed in utero to diethylstilbestrol, a non-steroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. In one of these studies, this risk was estimated as not greater than 4 per 1000 exposures. Furthermore, there are reports that a high percentage of such exposed women (from 30 to 90 percent) have been found to have vaginal adenosis, epithelial changes of the vagina and cervix. Although these reported changes are histologically benign, the investigators have not determined whether they are precursors of adenocarcinoma.

Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies in the offspring, including heart defects and limb reduction defects. One case control study estimated a 4.7 fold increased risk of limb reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 per 1000.

In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. OGEN has not been studied for these uses, and therefore should not be used during pregnancy. There is no evidence from well controlled studies that progestogens are effective for these uses.

If OGEN (estropipate tablets) is used during pregnancy, or if the patient becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus, and the question of continuation of the pregnancy should be addressed.

## **INDICATIONS AND USAGE**

The cyclic administration (See "DOSAGE AND ADMINISTRATION" section) of OGEN (estropipate tablets) is indicated for the treatment of estrogen deficiency associated with:

1. Moderate to severe vasomotor symptoms of menopause. (There is no evidence that estrogens are effective for nervous symptoms or depression which might occur during menopause, and they should not be used to treat these conditions.)
2. Atrophic vaginitis.
3. Kraurosis vulvae.
4. Female hypogonadism.
5. Female castration.
6. Primary ovarian failure.

**OGEN (ESTROPIRATE TABLETS) HAS NOT BEEN TESTED FOR EFFICACY FOR ANY PURPOSE DURING PREGNANCY. SINCE ITS EFFECT UPON THE FETUS IS UNKNOWN, IT CANNOT BE RECOMMENDED FOR ANY CONDITION DURING PREGNANCY (SEE BOXED WARNING).**

## **CONTRAINDICATIONS**

OGEN should not be used in women with any of the following conditions:

1. Known or suspected cancer of the breast.
2. Known or suspected estrogen-dependent neoplasia.
3. OGEN may cause fetal harm when administered to a pregnant woman. OGEN is contraindicated in women who are or may become pregnant (See Boxed Warning).
4. Undiagnosed abnormal genital bleeding.
5. Active thrombophlebitis or thromboembolic disorders.
6. A past history of thrombophlebitis, thrombosis, or thromboembolic disorders associated with previous estrogen use.

## **WARNINGS**

1. **Induction of malignant neoplasms.** Long-term continuous administration of natural and synthetic estrogens in certain animal species has been reported by some investigators to increase the frequency of carcinomas of the breast, cervix, vagina, and liver. There is now evidence that estrogens increase the risk of carcinoma of the endometrium in humans. (See Boxed Warning).

At the present time there is no conclusive evidence that estrogens given to postmenopausal women increase the risk of cancer of the breast. There are, however, a few retrospective studies which suggest a small but statistically significant increase in the risk factor for breast cancer among these women. Therefore, caution should be exercised when administering estrogens to women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease, or abnormal mammograms. Careful breast examinations should be performed periodically.

2. **Gall bladder disease.** A recent study has reported a 2 to 3-fold increase in the risk of surgically confirmed gall bladder disease in women receiving postmenopausal estrogens, similar to the 2-fold increase previously noted in users of oral contraceptives. In the case of oral contraceptives, the increased risk appeared after two years of use.

3. **Effects similar to those caused by estrogen-progestogen oral contraceptives.** There are several serious adverse effects of oral contraceptives, most of which have not, up to now, been documented as consequences of postmenopausal estrogen therapy. This may reflect the comparatively low doses of estrogen used in postmenopausal women. It would

be expected that the larger doses of estrogen used to treat postpartum breast engorgement would be more likely to result in these adverse effects, and, in fact, it has been shown that there is an increased risk of thrombosis in women receiving estrogens for postpartum breast engorgement.

a. **Thromboembolic disease.** It is now well established that users of oral contraceptives have an increased risk of various thromboembolic and thrombotic vascular diseases, such as thrombophlebitis, pulmonary embolism, stroke, and myocardial infarction. Cases of retinal thrombosis, mesenteric thrombosis, and optic neuritis have been reported in oral contraceptive users. There is evidence that the risk of several of these adverse reactions is related to the dose of the drug. An increased risk of post-surgery thromboembolic complications has also been reported in users of oral contraceptives. If feasible, estrogen should be discontinued at least 4 weeks before surgery of the type associated with an increased risk of thromboembolism; it should also be discontinued during periods of prolonged immobilization.

While an increased rate of thromboembolic and thrombotic disease in postmenopausal users of estrogens has not been found this does not rule out the possibility that such an increase may be present or that subgroups of women who have underlying risk factors or who are receiving relatively large doses of estrogens may have increased risk. Therefore estrogens should not be used in persons with active thrombophlebitis or thromboembolic disorders, and they should not be used in persons with a history of such disorders in association with estrogen use. They should be used with caution in patients with cerebral vascular or coronary artery disease and only for those in whom estrogens are clearly needed.

Large doses of estrogen (5 mg conjugated estrogens per day), comparable to those used to treat cancer of the prostate and breast, have been shown in a large prospective clinical trial in men to increase the risk of nonfatal myocardial infarction, pulmonary embolism and thrombophlebitis. When estrogen doses of this size are used, any of the thromboembolic and thrombotic adverse effects associated with oral contraceptive use should be considered a clear risk.

b. **Hepatic adenoma.** Benign hepatic adenomas appear to be associated with the use of oral contraceptives. Although benign, and rare, these may rupture and cause death through intraabdominal hemorrhage. Such lesions have not yet been reported in association with other estrogen or progestogen preparations but should be considered in estrogen users having abdominal pain and tenderness, abdominal mass, or hypovolemic shock. Hepatocellular carcinoma has also been reported in women taking estrogen-containing oral contraceptives. The relationship of this malignancy to these drugs is not known at this time.

c. **Elevated blood pressure.** Increased blood pressure is not uncommon in women using oral contraceptives. There is now a report that this may occur with use of estrogens in the menopause and blood pressure should be monitored with estrogen use, especially if high doses are used.

d. **Glucose tolerance.** A worsening of glucose tolerance has been observed in a significant percentage of patients on estrogen-containing oral contraceptives. For this reason, diabetic patients should be carefully observed while receiving estrogen.

e. **Hypercalcemia.** Administration of estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases. If this occurs, the drug should be stopped and appropriate measures taken to reduce the serum calcium level.

## **PRECAUTIONS**

### **A. General Precautions.**

1. A complete medical and family history should be taken prior to the initiation of any estrogen therapy. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen, and pelvic organs, and should include a Papanicolaou smear. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination being performed.

2. **Fluid retention.** Estrogens may cause some degree of fluid retention. Therefore, patients with conditions such as epilepsy, migraine, and cardiac or renal dysfunction, which might be influenced by this factor, require careful observation.

3. Certain patients may develop undesirable manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding, mastodynia, etc.

4. Oral contraceptives appear to be associated with an increased incidence of mental depression. Although it is not clear whether this is due to the estrogenic or progestogenic component of the contraceptive, patients with a history of depression should be carefully observed.

5. Preexisting uterine leiomyomata may increase in size during estrogen use.

6. The pathologist should be advised of the patient's use of estrogen therapy when relevant specimens are submitted.

7. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving estrogen-containing oral contraceptive therapy. If jaundice develops in any patient receiving estrogen, the medication should be discontinued while the cause is investigated.

8. Estrogens may be poorly metabolized in patients with impaired liver function and they should be administered with caution in such patients.

9. Because estrogens influence the metabolism of calcium and phosphorus, they should be used with caution in patients with metabolic bone diseases that are associated with hypercalcemia or in patients with renal insufficiency.

B. **Information for the Patient.** See text of Patient Package Insert which appears after PHYSICIAN REFERENCES.

C. **Drug Interactions.** The concomitant use of any drugs which can induce hepatic microsomal enzymes with estrogens may produce estrogen levels which are lower than would be expected from the dose of estrogen administered.

The use of broad spectrum antibiotics which profoundly effect intestinal flora may influence the absorption of steroidal compounds including the estrogens.

Diabetics receiving insulin may have increased insulin requirements when receiving estrogens.

Laboratory Test Interference. Certain endocrine and liver function tests may be affected by estrogen-containing oral contraceptives. The following similar changes may be expected with larger doses of estrogen:

- a. Increased sulfobromophthalein retention.
- b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.
- c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by PBI, T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered.
- d. Abnormal glucose tolerance test results.
- e. Decreased pregnandiol excretion.
- f. Reduced response to metoprolol test.
- g. Reduced serum folate concentration.
- h. Increased serum triglyceride and phospholipid concentration.

D. **Carcinogenesis.** Studies have shown an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for prolonged periods (see Boxed Warning). At the present time there is no conclusive evidence that estrogens given to postmenopausal women increase the risk of cancer of the breast. There are, however, a few retrospective studies which suggest a small but statistically significant increase in the risk factor for breast cancer among these women. (See "WARNINGS" section.)

E. **Pregnancy.** Pregnancy Category X. See "CONTRAINDICATIONS" section and Boxed Warning.

F. **Nursing Mothers.** Estrogens have been reported to be excreted in human breast milk. Caution should be exercised when OGEN is administered to a nursing woman.

G. **Pediatric Use.** Because of the effects of estrogens on epiphyseal closure, they should be used judiciously in young patients in whom bone growth is not complete.

## **ADVERSE REACTIONS**

(See Warnings regarding reports of possible induction of neoplasia, unknown effects upon the fetus, increased incidence of gall bladder disease, and adverse effects similar to those of oral contraceptives, including thromboembolism.) The following additional adverse reactions in decreasing order of severity within each category have been reported with estrogenic therapy, including oral contraceptives:

1. **Genitourinary system.**  
Increase in size of uterine fibromyomata.  
Vaginal candidiasis.  
Cystitis-like syndrome.  
Dysmenorrhea.  
Amenorrhea during and after treatment.  
Change in cervical eversion and in degree of cervical secretion.  
Breakthrough bleeding, spotting, change in menstrual flow.  
Premenstrual-like syndrome.
2. **Breast.**  
Tenderness, enlargement, secretion.
3. **Gastrointestinal.**  
Cholestatic jaundice.  
Vomiting, nausea.  
Abdominal cramps, bloating.
4. **Skin.**  
Hemorrhagic eruption.  
Erythema nodosum.  
Erythema multiforme.  
Hirsutism.  
Chloasma or melasma which may persist when drug is discontinued.  
Loss of scalp hair.
5. **Eyes.**  
Steepening of corneal curvature.  
Intolerance to contact lenses.
6. **CNS.**  
Chorea.  
Mental depression.  
Migraine, dizziness, headache.
7. **Miscellaneous.**  
Aggravation of porphyria.  
Edema.  
Reduced carbohydrate tolerance.  
Increase or decrease in weight.  
Changes in libido.

## **OVERDOSAGE**

Numerous reports of ingestion of large doses of estrogen-containing oral contraceptives by young children indicate that serious ill effects do not occur. Overdosage of estrogen may cause nausea and withdrawal bleeding may occur in females.

## **DOSAGE AND ADMINISTRATION**

### **1. Given cyclically for short-term use.**

For treatment of moderate to severe vasomotor symptoms, atrophic vaginitis, or kraurosis vulvae associated with the menopause.

The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible.

Administration should be cyclic (e.g., 3 weeks on and 1 week off).

Attempts to discontinue or taper medication should be made at 3 to 6 month intervals.

Usual dosage ranges:

**Vasomotor symptoms.**—One OGEN .625 Tablet to one OGEN 5 Tablet per day. The lowest dose that will control symptoms should be chosen. If the patient has not menstruated within the last two months or more, cyclic administration is started arbitrarily. If the patient is menstruating, cyclic administration is started on day 5 of bleeding.

**Atrophic vaginitis and kraurosis vulvae.**—One OGEN .625 Tablet to one OGEN 5 Tablet daily, depending upon the tissue response of the individual patient. The lowest dose that will control symptoms should be chosen. Administer cyclically.

### **2. Given cyclically.**

Female hypogonadism; female castration; primary ovarian failure.

Usual dosage ranges:

**Female hypogonadism.**—A daily dose of one OGEN 1.25 Tablet to three OGEN 2.5 Tablets may be given for the first three weeks of a theoretical cycle, followed by a rest period of eight to ten days. The lowest dose that will control symptoms should be chosen. If bleeding does not occur by the end of this period, the same dosage schedule is repeated. The number of courses of estrogen therapy necessary to produce bleeding may vary depending on the responsiveness of the endometrium. If satisfactory withdrawal bleeding does not occur, an oral progestogen may be given in addition to estrogen during the third week of the cycle.

**Female castration and primary ovarian failure.**—A daily dose of one OGEN 1.25 Tablet to three OGEN 2.5 Tablets may be given for the first three weeks of a theoretical cycle, followed by a rest period of eight to ten days. Adjust dosage upward or downward according to severity of symptoms and response of the patient. For maintenance, adjust dosage to lowest level that will provide effective control.

Treated patients with an intact uterus should be monitored closely for signs of endometrial cancer and appropriate diagnostic measures should be taken to rule out malignancy in the event of persistent or recurring abnormal vaginal bleeding.

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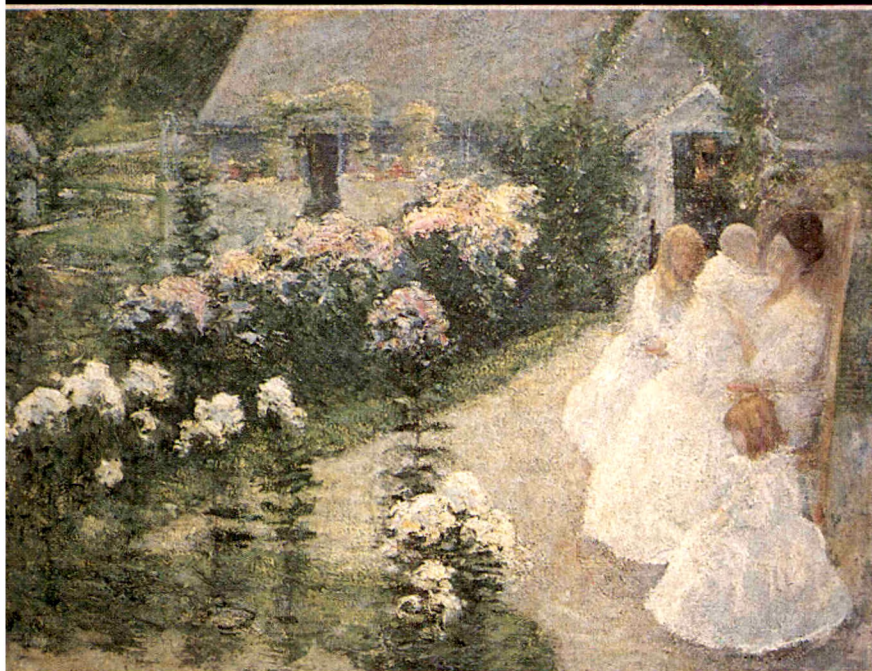
OGEN (estropipate tablets, USP) is supplied as OGEN .625 (0.75 mg estropipate), yellow tablets, NDC 0074-3943-04; OGEN 1.25 (1.5 mg estropipate), peach-colored tablets, NDC 0074-3946-04; OGEN 2.5 (3 mg estropipate), blue tablets, NDC 0074-3951-04; and OGEN 5 (6 mg estropipate), light green tablets, NDC 0074-3958-13. Tablets of all four dosage levels are standardized to provide uniform estrone activity and are grooved (Divide-Tab®) to provide dosage flexibility. All tablet sizes of OGEN are available in bottles of 100.

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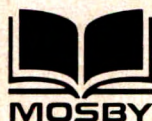
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**TOP:** John Henry Twachtman, **ON THE TERRACE**, c. 1890-1900. Canvas, approx. 64.8 x 76.2 cm. National Museum of American Art, Smithsonian Institution, Washington, D.C., Gift of John Gellatly.

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# Fibrocystic breast disease: Pathophysiology, pathomorphology, clinical picture, and management

Helmuth Vorherr, M.D.

Albuquerque, New Mexico

The pathophysiology of fibrocystic breast disease is determined by estrogen predominance and progesterone deficiency that result in hyperproliferation of connective tissue (fibrosis), which is followed by facultative epithelial proliferation; the risk of breast cancer is increased twofold to fourfold in these patients. The clinical correlate of fibrocystic disease is reflected by breast and axillary pain or tenderness in response to development of fibrocystic plaques, nodularity, macrocysts, and fibrocystic lumps. The disease progresses with advancing premenopausal age and is most pronounced in women during their 40s. Fibrocystic changes regress during the postmenopausal period. Medical treatment of fibrocystic disease is accomplished: (1) by suppression of ovarian estrogen secretion with a low-estrogen oral contraceptive, whereby the action of estrogen on breast tissues is opposed by the oral contraceptive's progestin component (19-nortestosterone derivatives), or (2) by cyclic administration of a progestogen (progesterone, medroxyprogesterone acetate) that modulates the mammary effects of estrogen. These treatment modalities are equally as effective as or superior to danazol therapy, which entails side effects in the majority of patients. Adjuvant therapy of fibrocystic breast disease with vitamin E is of value in patients with borderline or abnormal lipid profiles (low plasma levels of high-density lipoprotein and high plasma levels of low-density lipoprotein). With thorough diagnostic evaluation, appropriate medication, and close follow-up, treatment success can be achieved in almost every patient. Needle aspiration biopsy should be performed in patients with macrocysts and whenever clinical, ultrasonic, and/or mammographic examinations are suspicious for carcinoma. Patients at high risk of breast cancer (breast cancer in mother and/or sister) should have clinical examinations at 4- to 6-month intervals and mammography every 1 to 2 years; needle aspiration should be performed when the slightest suspicion arises. Fibrocystic breast disease is not a "harmless nondisease" but a distinct clinical entity that requires treatment to bring about relief to the patient, to reduce the incidence of breast surgical procedures, and to diminish the risk of breast cancer. (AM J OBSTET GYNECOL 1986;154:161-79.)

**Key words:** Fibrocystic disease, pathobiology, management

Benign breast disease encompasses a variety of disorders, mostly made up of fibrocystic breast disease and its various subtypes (Table I).

Early diagnosis and medical treatment of fibrocystic disease can arrest and reverse fibrocystic changes, thus sparing the patient an operation. Untreated, fibrocystic breast disease may progress to breast lumpiness which at any age is associated with the possibility of cancer. Breast biopsies are increasingly performed in patients whose mothers and/or sisters had fibrocystic disease.

The risk of breast cancer is increased twofold in pa-

tients with preceding breast biopsy. Fifty percent to 75% of all breast biopsies are performed because of fibrocystic breast disease; approximately 25% of biopsies in patients with fibrocystic disease reveal carcinoma. In 50% to 80% of patients with a breast lump, the mass is discovered by the patient. A carcinoma is found in one of four to five breast biopsy specimens. Multiple breast lumps as observed in fibrocystic disease that has progressed are usually benign; a single, hard, nontender, irregular lump is more likely to be cancerous. However, thorough diagnostic evaluation is required in any patient with breast lumps.

### Pathophysiology of fibrocystic disease

Reproductive hormones (estrogens, prolactin), thyroid hormones, and methylxanthines have been as-

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**Table I.** Fibrocystic disease and related pathology in premenopausal women: Incidence and pathomorphology

<i>Types</i>	<i>Percent incidence</i>	<i>Peak incidence (yr)</i>	<i>Pathomorphology</i>
Fibrocystic disease	20-40	35-45	Stromal fibrosis, ductular branching, intraductal epithelial proliferation, cyst formation
Mazoplasia	20	35-50	Focal ductular-alveolar hyperplasia, stromal edema; epithelial desquamation, secretory retention, and induration (nodularity) of terminal ductal segments; lymphocyte infiltration; circumductal fibrosis
Sclerosing adenosis	7-10	30-40	Proliferation of ductular-alveolar epithelium (lobular hyperplasia) and connective tissue (fibroelastosis) followed by epithelial atrophy and sclerosis
Intraductal proliferation	10-15	35-45	Intraductal epithelial hyperplasia (papillomatosis)
Macrocyts	10-20	35-45	Secretory retention and cyst formation subsequent to terminal ductal-lobular obstruction by stromal fibrosis
Fibroadenoma	15-20	20-25	Proliferation of intralobular connective tissue (fibrosis) and glandular epithelium (adenosis)
Adenoma	1-2	20-30	Focal, encapsulated tumor-forming adenosis of ductal-alveolar structures (tubular or tubular-alveolar differentiation)

sociated with the development of fibrocystic breast disease.

**Estrogens and progesterone.** Estrogen predominance over progesterone is considered causative in the development of fibrocystic breast disease<sup>1</sup> (Fig. 1). Progesterone counteracts the proliferative effects of estrogen and brings about epithelial differentiation and reduction of mitoses.<sup>2, 3</sup> Patients with premenstrual tension syndrome have estrogen predominance over progesterone and are more likely to develop fibrocystic breast disease. Such patients experience cyclically each month, near the time of menstruation, estrogen-induced symptoms such as irritability, anxiety, hostility; nervous tension, insomnia; abdominal bloating; pelvic pain; swelling of fingers, dorsal hands, and ankles; and breast pain, tenderness, and engorgement. Estrogens increase water retention in connective tissue (interlobular edema), while progesterone modulates the local effects of estrogen and also facilitates renal excretion of sodium and water by antagonizing the action of aldosterone on the distal nephron.

In patients with fibrocystic disease, serum estrogen levels are normal or increased, the luteal phase progesterone level is decreased to one third of normal, and the luteal phase is shortened. Estradiol levels in breast tissue have been found increased. Almost 70% of patients with fibrocystic disease have either corpus luteum deficiency or anovulation.<sup>4,6</sup> Patients with progesterone deficiency seem to carry a fivefold higher risk of premenopausal breast cancer.<sup>7</sup>

**Prolactin.** The plasma prolactin level has been found slightly increased in approximately one third of women with fibrocystic disease. An increase in prolactin levels has been explained by the predominance of estrogens and luteal deficiency of progesterone; estrogens enhance pituitary prolactin secretion. Melis et al.<sup>5</sup> ob-

served hyperprolactinemia (prolactin levels of 30 to 40 ng/ml of plasma) in 15% of patients with fibrocystic disease. According to Peters et al.,<sup>8</sup> 39% of patients with fibrocystic disease had prolactin levels of 15 to 30 ng/ml; 21% of the control subjects had similar levels. Therefore, it has been suggested that prolactin is of pathogenic importance in fibrocystic breast disease.<sup>8</sup> It is generally accepted, however, that serum prolactin levels are normal in patients with fibrocystic disease and that prolactin is not associated with the development of fibrocystic disease or breast cancer.<sup>9</sup> Rather, women with prolactin levels that are 10 or more times normal, as observed during pregnancy and lactation, are protected from fibrocystic disease and breast cancer.<sup>9</sup> In the state of hyperprolactinemia, ovarian estrogen secretion is decreased and the mammary epithelium is differentiated for milk synthesis and milk secretion. No or only little epithelial and stromal proliferation occurs and fibrocystic disease is counteracted.<sup>3</sup>

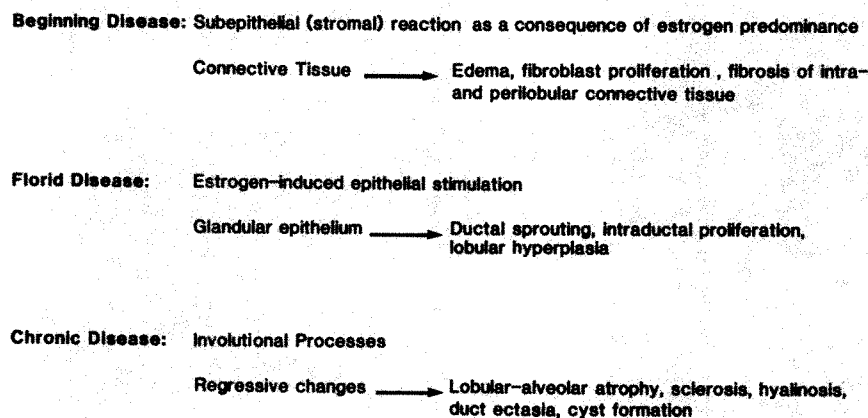
We found that estrogen treatment, in hypophysectomized rats lacking prolactin, induces fibrocystic mammary changes that are greater than those in controls.

**Thyroid hormones.** It is assumed that fibrocystic breast disease is more likely to develop in patients with hyperthyroidism because of thyroid hormone-induced hypersensitivity of mammary tissues to estrogens. However, patients with fibrocystic disease have been found hypothyroid and thyroid hormone has been recommended for treatment.<sup>4</sup> Suboptimal levels of thyroid hormones are thought to sensitize mammary epithelial cells to prolactin stimulation.<sup>10</sup>

Thyroid hormone levels were consistently found to be higher in breast cyst fluid than in plasma, but this may represent a mere consequence of fibrocystic changes.<sup>10</sup>

Despite many studies, it is presently not known





**Fig. 1.** The pathophysiology of fibrocystic breast disease entails an initial stromal reaction. Florid disease is evidenced by proliferation of connective and epithelial tissues. In chronic disease, regressive glandular-epithelial changes predominate.

whether thyroid hormone has a promoting or a protecting effect on fibrocystic disease and/or breast cancer.<sup>9</sup>

**Methylxanthines.** Intake of methylxanthines (coffee, tea, cola drinks, chocolate) has been associated with the development of fibrocystic breast disease.<sup>11</sup> It has been claimed that methylxanthines stimulate cyclic adenosine monophosphate and guanosine monophosphate in breast tissues and thus cause fibrocystic disease. After elimination of caffeine from the diet, 13 of 20 patients (65%) "experienced complete disappearance of all palpable breast nodules, pain, tenderness, and nipple discharge" within 1 to 6 months.<sup>11</sup> However, several other studies revealed that methylxanthine intake is not associated with fibrocystic breast disease.

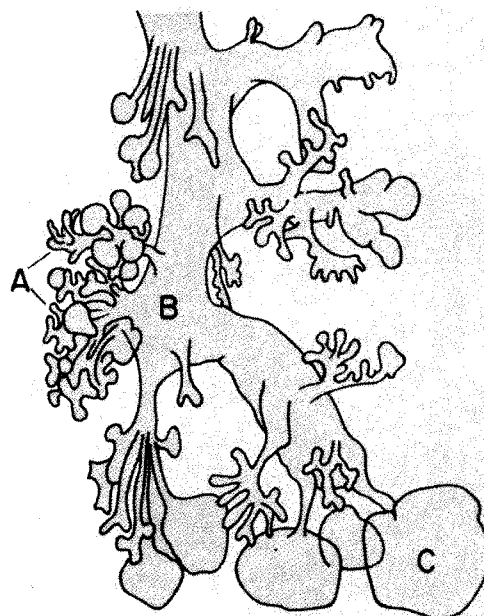
Avoidance of coffee, tea, cola drinks, and chocolate appears to have no influence on the development and course of fibrocystic breast disease in our experience with almost 400 patients.

**Trauma.** Trauma to the breast (mastitis, mammary abscess, breast injury) does not appear to be an etiologic factor in the development of benign and malignant breast disease.<sup>9</sup> Unfortunately, sometimes the belief that a lump, later diagnosed as cancerous, is due to injury has led to delay of diagnosis and opportunity for early treatment.

It is not clear to what extent, if any, impingements on a woman's breast for sexual pleasure have an influence on development and/or course of fibrocystic disease.

### Pathomorphology of fibrocystic disease

Estrogens stimulate proliferation of connective and epithelial tissues while progesterone modulates the effects of estrogen, bringing about normal lobular-ductular-alveolar development.<sup>2, 5, 12</sup> In addition to stromal stimulation (fibroblast hyperactivity, edema), estrogens cause proliferation of ductal-alveolar epithelium.<sup>1, 13</sup>



**Fig. 2.** Scheme of pathomorphology of fibrocystic disease (drawn from thick slices). The epithelial pathomorphogenesis of fibrocystic disease entails: A, ductal-lobular proliferation; B, duct dilation and elongation; C, terminal ductal cyst formation. (From Tanaka Y, Oota K. A stereomicroscopic study of the mastopathic human breast. I. Three-dimensional structures of abnormal duct evolution and their histologic entity. *Virchows Arch [A]* 1970;349:195-214.)

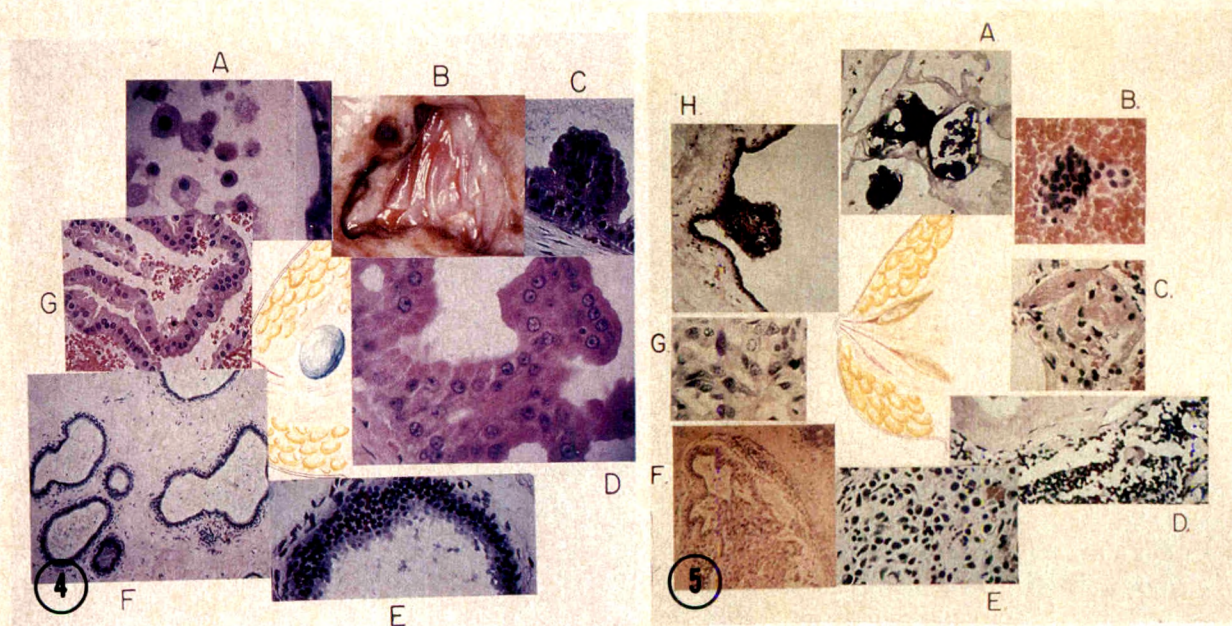
Usually, fibrosis precedes and predominates epithelial proliferation (Fig. 1).

In the histologic preparation, the polymorphism of fibrocystic disease is documented by fibrosis, cyst formation, epithelial proliferation, and lobular-alveolar atrophy, processes that may be coexisting in the same biopsy specimen. Accordingly, fibrocystic disease entails simultaneous progressive and regressive changes.<sup>14</sup> Ductular branching, intraductal epithelial proliferation (papillomatosis), lobular hyperplasia, and proliferation





**Fig. 3.** Cystic disease: Multiple macrocysts in the mastectomy specimen of a patient with fibrocystic disease. (From Bässler R. Pathologie der Brustdrüse. Berlin: Springer-Verlag, 1978.)



**Fig. 4.** Pathomorphology of cyst formation. A: Phagocytosing histiocytes (foam cells) in cyst fluid; B, C, and D: intracystic papillomatosis (D shows apocrine metaplasia); E: intracystic proliferation; F: microcysts with a single epithelial lining; G: apocrine metaplasia in multihole needle aspirate from a cyst.

**Fig. 5.** Pathomorphology of duct ectasia. A: Intraductal and periductal calcification; B: intraductal proliferation; C: periductal fibrosis; D and E: periductal round cell infiltration (lymphocytes, plasma cells); F: duct ectasia with intraductal epithelial proliferation and carcinoma in situ; G: magnification of F (~400-fold of the original); H: postmenopausal duct ectasia with intraductal papilloma.

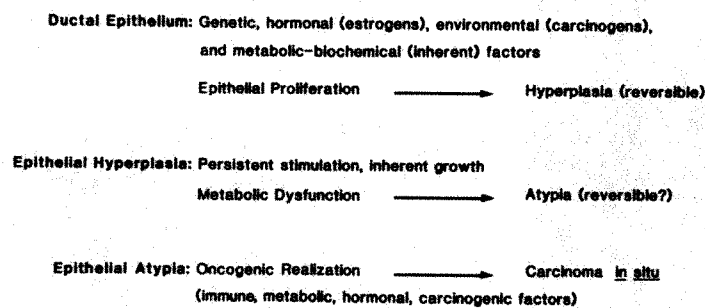
of intralobular connective tissue may undergo regressive changes such as adenofibrosis, sclerosing adenosis, duct dilation, cyst formation, and calcification.<sup>13-15</sup> Loss of parenchymal elements (ductules, alveoli) with intralobular and periductal fibrosis is encountered in chronic disease.

**Cyst formation.** As a consequence of obstruction by stromal fibrosis and persisting ductular-alveolar secretion, secretory material is retained, leading to dilation of terminal ducts (duct ectasia) and alveoli with cyst formation<sup>14, 16</sup> (Fig. 2). In 20% to 40% of patients with

fibrocystic disease, gross cyst formation is observed<sup>1</sup> (Fig. 3).

Cyst fluid (pH 7.1 to 7.4) contains proteins, hormones (prolactin, estrogens, androgens, human chorionic gonadotropin, human growth hormone, follicle-stimulating hormone, luteinizing hormone), glucose, minerals, and cholesterol.<sup>5, 9</sup> Also, ductular-alveolar breast epithelium is capable of synthesizing androgens and estrogens from pregnenolone.<sup>9</sup> Depending on the type and stage of fibrocystic disease, cyst content may be milky-creamy, serous, yellow, brown-green, green-blue,





**Fig. 6.** Pathophysiology of epithelial proliferation. Hormonal (estrogens) and other factors stimulate ductal epithelial proliferation leading to epithelial hyperplasia and possibly, via epithelial atypia, to carcinoma in situ.

**Table II.** Fibrocystic disease with epithelial proliferation: Incidence and risk of breast cancer

Fibrocystic disease	Incidence (%)	Risk of breast cancer
All types (with and without epithelial proliferation)	—	2- to 3-fold increased
No epithelial proliferation	70	0- to 2-fold increased
Epithelial proliferation	20	2- to 4-fold increased
Atypical intraductal proliferation	10	5-fold increased
Epithelial atypia plus family history of breast cancer	—	11-fold increased
Atypical lobular proliferation	1-2	4- to 6-fold increased

ink black, serosanguineous, or sanguineous. Abacterial inflammatory reactions to fibrocystic processes, in conjunction with decomposition and degradation of cyst contents, lead to color changes of cyst fluid. Fluid of cysts and dilated ductules may be resorbed in part by histiocytes, which leads to a more creamy content with predominance of foam cells. Calcification of cyst content occurs in approximately 25% of patients.<sup>13</sup> Serosanguineous or sanguineous cyst fluid is derived from intracystic papilloma (Fig. 4) or rarely (1%) from carcinoma.

Macrocyts (>1 cm in diameter) represent an advanced form of fibrocystic disease.<sup>16</sup> They develop in women mainly in their forties and, depending on the degree of fluid filling and pericystic fibrosis, appear softer or harder.

**Duct ectasia.** Approximately 25% of those premenopausal patients who do develop peripheral mammary duct dilation do so because of fibrocystic disease. Here, terminal ducts become obstructed through fibrotic tissue strands, leading to accumulation of secretory material and ectasia (Fig. 5).

Dilation of larger mammary ducts is observed in 60% to 70% of postmenopausal women as the consequence of glandular involution and secretory retention. Us-

**Table III.** Fibrocystic disease-related pathology

*Papilloma*

In 50% subareolar mass and/or bloody nipple discharge  
In 20% breast pain (ductal stretching)  
Solitary papilloma arises mainly from retroareolar ducts  
Multiple papilloma arises mainly from peripheral ducts (association with carcinoma)

*Adenoma*

Mainly during pregnancy due to focal estrogen hyperstimulation

*Fibroadenoma*

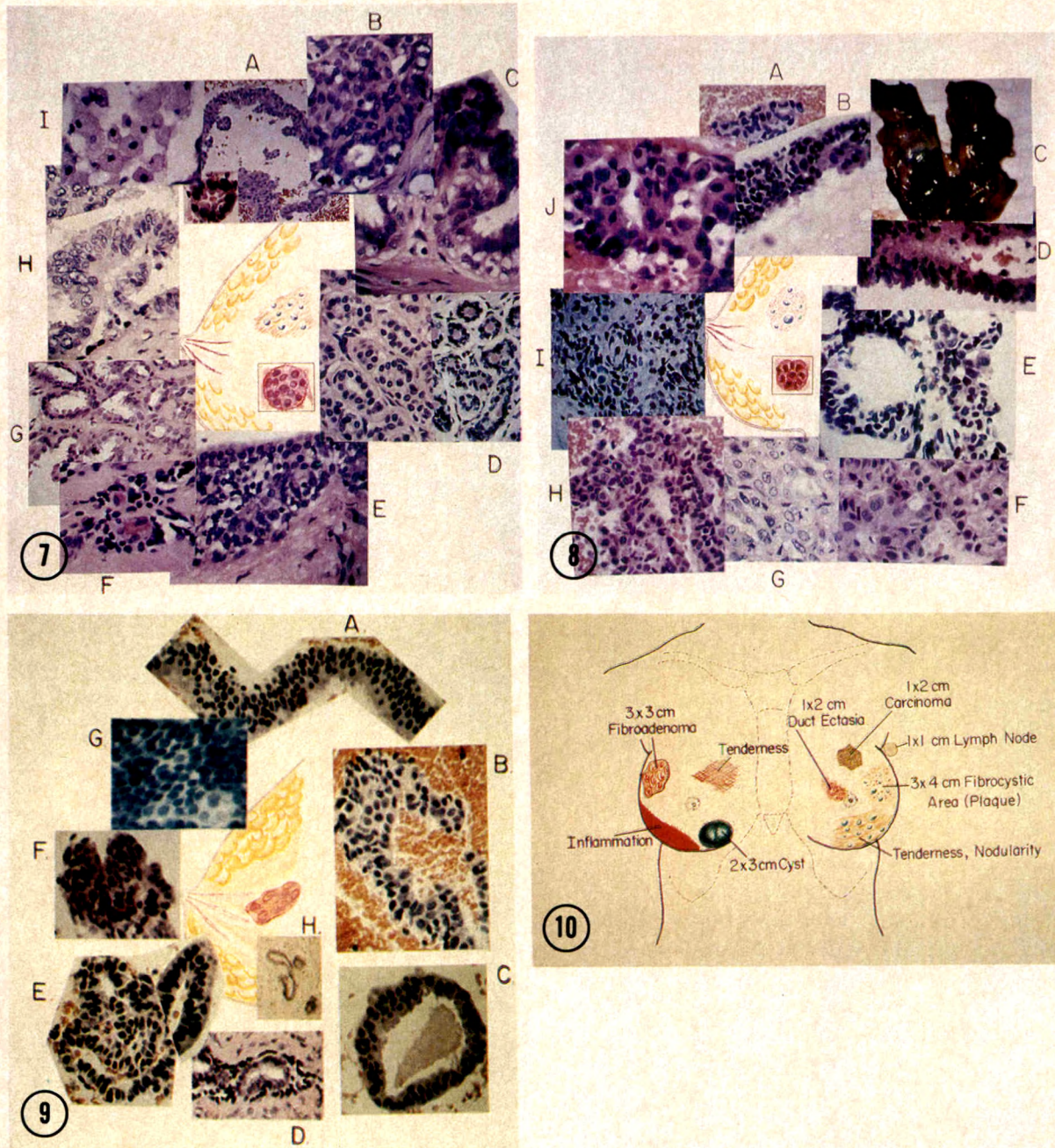
Fibroepithelial tumor (multiple: 10% to 20%; bilateral: 3% to 5%; 20% to 30% of breast disease below age 25)  
Initial stromal edema followed by abnormal duct proliferation  
Association with apocrine metaplasia (20% to 30%) and sclerosing adenosis (20%)  
Malignant degeneration: <1% (mainly cystosarcoma phyllodes)

*Sclerosing adenosis*

Tumor-forming type (age 30) and diffuse type (age 30 to 50)  
The only benign condition with epithelial invasion into stroma  
Three phases: (1) epitheliosis, (2) focal sclerosis, (3) loss of lobular epithelium  
"Radial scar": epithelial cells within fibrotic tissue resembling scirrhous carcinoma

ally, subareolar ducts become cylindrically dilated and filled with a yellow-white creamy material, presenting clinically as a plaque or a lumplike structure. The viscous intraductal material contains fat droplets, phagocytosing histiocytes (foam cells), and plasma cells. In 20% to 30% of patients nipple discharge (foam cells) and microcalcifications (ductal lumen and wall, Fig. 5, A) are observed. Subareolar duct ectasia may extend peripherally. In many patients duct ectasia is painful because of secretory retention, ductal wall stretching, and neural irritation. When the ductal wall tears and contents reach the stroma, an abacterial (chemical), very painful periductal inflammatory reaction (plasma cell mastitis, Fig. 5, D and E) may occur with infiltration of plasma cells, histiocytes, lymphocytes, and mast cells (complicated duct ectasia).





Figs. 7 to 10. For legends see opposite page.

#### Pathobiology of ductal and lobular epithelial proliferation

Intraductal proliferation (epithelial hyperplasia, papillomatosis) and lobular proliferation (hyperplasia of acinar epithelium) are considered processes of estrogen hyperstimulation and are observed in one third of patients with fibrocystic disease (Fig. 6). Intraductal epithelial proliferation is diagnosed in 60% of patients with fibrocystic disease in whom multihole needle aspiration<sup>17, 18</sup> is performed; in one third of these patients atypia exists. Surgical excision is indicated in 20%

of patients who have undergone multihole needle aspiration. The pathologic features include intraductal proliferation in almost all of these patients; in half we find epithelial atypia.

Epithelial hyperplasia of medium-sized and smaller ducts and of lobules is often accompanied by apocrine metaplasia, cyst formation, sclerosing adenosis, and papillomatosis (Fig. 7).

Approximately 10% of breast masses are due to intraductal papilloma, which is observed most often in women aged 40 to 50. In half of these patients



**Table IV.** Fibrocystic breast disease: Clinical picture, course, symptoms

<i>Breast pain</i>
In more than 50% of patients pain is bilateral (predominantly upper outer quadrants)
In 15% to 30% of patients pain radiates into axilla
<i>Nipple secretion</i>
In 20% to 40% of patients with fibrocystic disease
In 2% to 3% of patients carcinoma is diagnosed
<i>Bloody nipple secretion</i>
In 50% to 60% intraductal proliferation (papilloma) is the cause
In 30% to 40% carcinoma is the cause (after age 50: in 64% carcinoma is the cause)
<i>Clinical course</i>
Phase 1: Moderate stromal fibrosis, beginning hardening of breast tissues, and premenstrual breast tenderness
Phase 2: Progressive fibrosis (increased hardening and breast tenderness, cyst formation, moderate nodularity)
Phase 3: Pronounced fibrosis and breast tenderness, macrocyst formation, development of pronounced nodularity and fibrocystic lumps ("lumpiness")

sanguineous discharge and/or an underlying lump exist.<sup>13</sup>

Intraductal epithelial proliferation is observed in 40% of older women, despite estrogen deficiency. It is possible that local ductal epithelial estrogen synthesis or, more so, conversion of androstenedione to estrone in periductal fat and diffusion of estrogen to adjacent ductal epithelium produce epithelial proliferation. Sixty percent of postmenopausal breast carcinomas possess estrogen receptors.

According to most authors, the risk of breast cancer is increased in patients with epithelial proliferation

**Table V.** Diagnosis of fibrocystic breast disease: Mammography, sonography, multihole needle aspiration

<i>Mammography</i>
Sensitivity for diagnosis of fibrocystic disease: 80%
Sensitivity for diagnosis of breast cancer: 60% to 95%
Macrocalcification (>0.5 mm diameter): predominant in patients with cysts, lipomas, sclerosing adenosis, and duct ectasia
Microcalcification (<0.5 mm diameter; usually 0.1 to 0.3 mm): in 40% to 60% of patients with fibrocystic disease, in 10% to 30% of patients with clinically occult carcinoma (half of these carcinomas are in situ)
<i>Sonography</i>
Sensitivity for diagnosis of fibrocystic disease: 80% to 90%
Sensitivity for diagnosis of macrocysts: 100%
Sensitivity for diagnosis of breast cancer: 60% to 80%
No detection of calcification
<i>Multihole needle aspiration</i>
Sensitivity for diagnosis of fibrocystic disease and epithelial proliferation: 80% to 90%
Sensitivity for diagnosis of breast cancer: 90% to 100%

(Table II). Page et al.<sup>19</sup> reported a threefold higher breast cancer risk in women with ductal hyperplasia who are over the age of 45.

Atypical proliferation (epithelial dysplasia) is considered a precancerous condition, that is, a transitional process to carcinoma in situ. Approximately 5% to 10% of patients with epithelial atypia develop carcinoma within 5 to 10 years (Fig. 8).

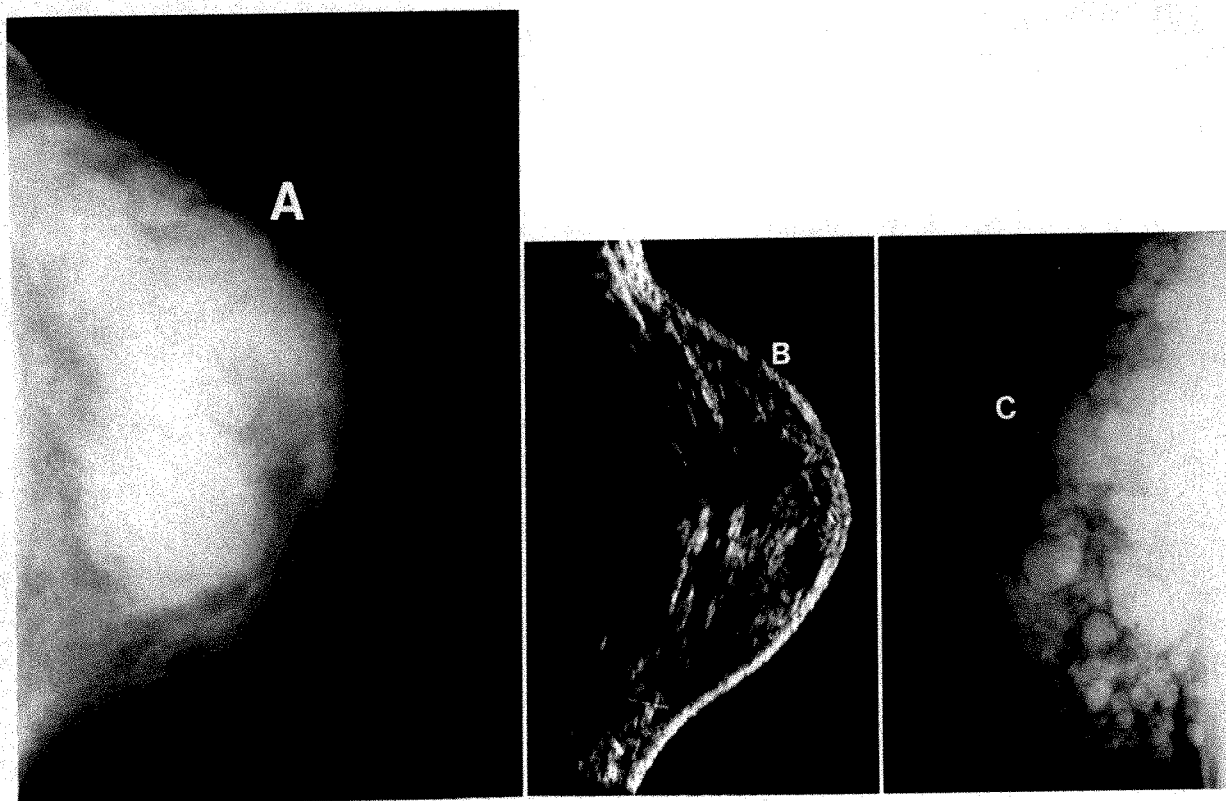
In 35% to 50% of patients with breast cancer, epithelial proliferation coexists. In 20% to 30% of these patients proliferation is atypical; even values of 60% to 70% have been reported.

**Fig. 7.** Pathomorphology of epithelial proliferation. *A, B, and C:* Intraductal proliferation (papillomatosis); *D:* lobular hyperplasia; *E and F:* sclerosing adenosis, stromal epitheliosis; *G:* subareolar papilloma in a postmenopausal woman; *H:* intraductal papillomatosis in a premenopausal patient; *I:* intraductal phagocytosing histiocytes (foam cells).

**Fig. 8.** Pathomorphology of atypical epithelial proliferation. *A and B:* Intraductal epithelial proliferation with moderate atypia (slight nuclear pleomorphism and hyperchromatism). *C and D:* Surgical specimen of fibrocystic lump with macrocysts (*C*); preceding multihole needle aspiration showing moderate atypia that was confirmed by surgical pathologic examination (*D*). *E and F:* Moderate to pronounced epithelial atypia in patients with fibrocystic disease. *G:* Moderate atypia adjacent to a focus of ductal carcinoma in situ (see Fig. 5, *F*). *H, and I:* Severe epithelial atypia with increase and variation in nuclear size, loss in even nuclear outline, and hyperchromatism. *J:* Severe atypia with transition to carcinoma. Histologic features of multihole needle aspiration include cells with macronuclei, polynucleation, nuclear pleomorphism and hyperchromatism, and rarely mitoses (surgical pathologic result: invasive ductal carcinoma).

**Fig. 9.** Pathomorphology of fibroadenoma. *A:* Strand of ductal epithelium of fibroadenoma; *B:* typical epithelial cells of fibroadenoma with increase in nuclear size and slight pleomorphism and hyperchromatism; *C:* fibroadenoma—duct with lactational changes; *D and E:* fibrous hyperplasia (*D*) with epithelial hyperplasia (*E*); *F:* fibroadenoma with intraductal proliferation; *G:* cytology (Papanicolaou staining) of fibroadenoma includes large cells with stroma; *H:* glandular and fibrous elements of fibroadenoma.

**Fig. 10.** Clinical breast pathology. Documentation of breast and axillary findings upon clinical examination.



**Fig. 11, A to C.** Mammography and sonography of patients with breast disease. **A:** Mammogram of a 43-year-old patient with fibrocystic disease, showing a DY (dysplastic) pattern with a large mass (lower part of picture). **B:** Sonogram of same patient showing a cyst (dark area). **C:** Mammogram of a 37-year-old patient with silicone injection showing multiple well-defined round densities.

#### **Fibrocystic disease—related pathologic conditions: Fibroadenoma, adenoma, papilloma, sclerosing adenosis**

Among benign breast tumors, fibroadenoma (63%), papilloma (16%), macrocysts (12%), tumor-forming sclerosing adenosis (2%), and adenomas (2%) are most frequent. Fibrocystic disease—related abnormality (Table III, Figs. 7 and 9) that develops under estrogen predominance is counteracted by intake of low-estrogen oral contraceptives whereby ovarian estrogen secretion is suppressed and the mammary effect of estrogen is modulated by the pill's progestin component.

#### **Clinical picture and course of fibrocystic disease**

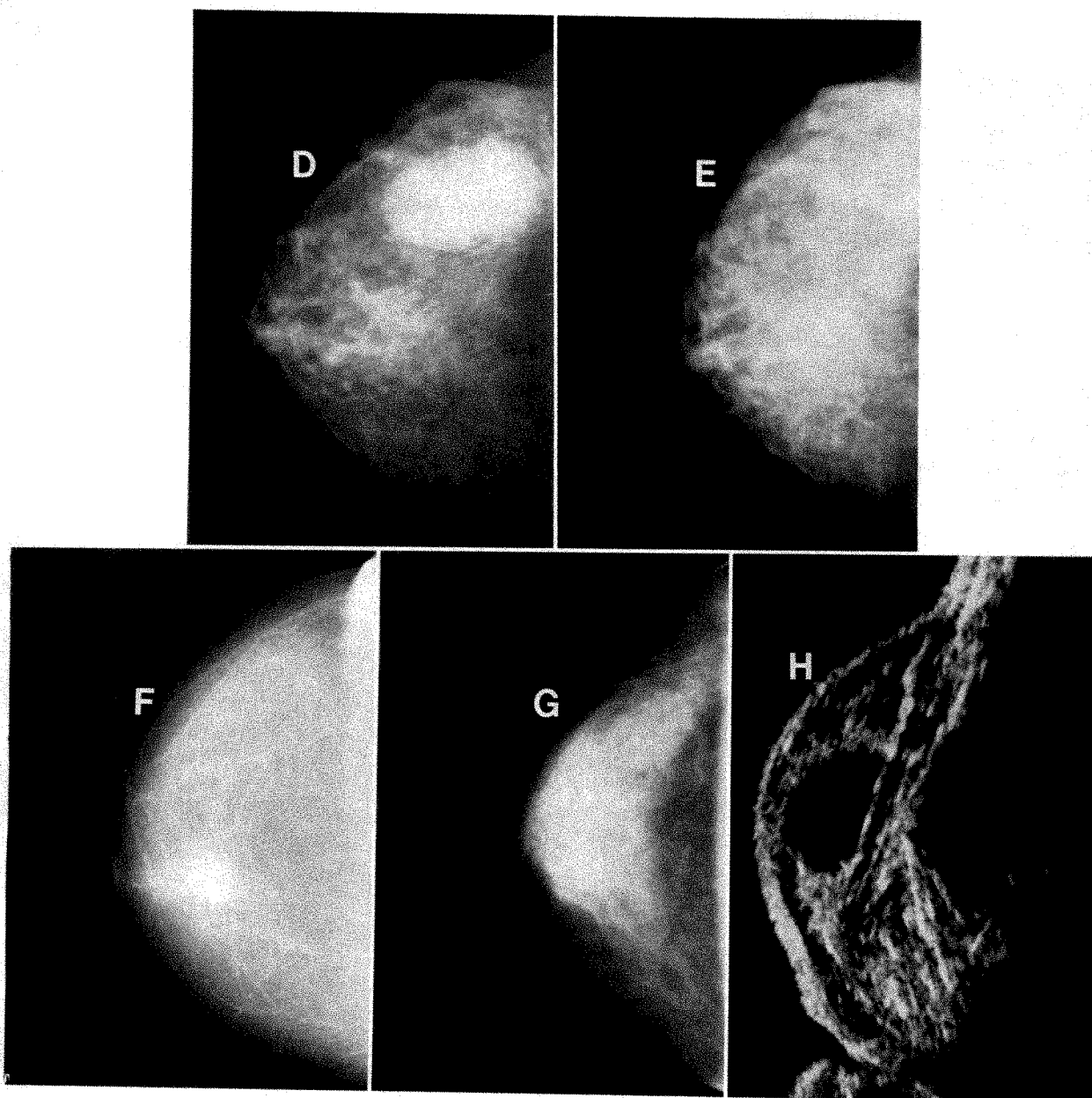
Fibrocystic disease represents a clinical problem in approximately 30% of patients. At the time of autopsy in 30% to 50% of premenopausal women fibrocystic disease is found; in one third of these cases intraductal epithelial proliferation exists.

Fibrocystic changes may involve only one breast or one quadrant. Usually the disease is multifocal and in more than half of the patients both breasts are involved (Table IV). With progressing disease and premenopausal age, the disease is bilateral in most patients. Pain and

tissue abnormalities are usually more pronounced in the left breast, and this may be attributed to the average larger size of the left over the right breast.<sup>2</sup> Also, despite seemingly diffuse fibrocystic disease as indicated by a dystrophic pattern (DY) in the mammogram, clinically, often one or more isolated fibrocystic changes can be palpated as a plaque or lumplike structure. For fibrocystic, tumorlike changes resembling fibroadenoma, the term hamartoma has been coined. Fibrocystic areas are usually very tender and bothersome to the patient.

Early fibrocystic manifestations may occur between the age of 20 and 25 years, but most patients (70% to 75%) are in their mid-30s and 40s. Predominantly afflicted are women with menstrual abnormalities, nulliparous women, patients with a history of spontaneous abortion(s), nonusers of oral contraceptives, and women with early menarche and late menopause. Our observations on 263 premenopausal patients with tubal sterilization (184 patients) and hysterectomy (79 patients) indicate that the risk of developing fibrocystic disease and breast cancer is increased as a consequence of ovarian dysfunction with a predominant estrogen effect on breast tissues.

Clinically, three phases of fibrocystic disease can be

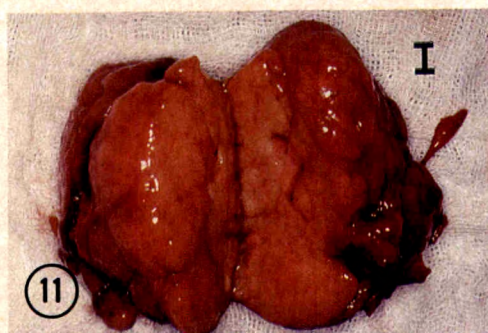


**Fig. 11, D to H.** Mammography and sonography of patients with breast disease. *D*: Mammogram of a 52-year-old patient with fibrocystic disease showing a mass 4 by 6 cm in diameter. Removal of 15 ml of greenish fluid. Aspiration histology revealed intraductal epithelial proliferation with slight to moderate atypia. *E*: Same patient as *D*. Mammogram 6 months later showing a remaining asymmetric ill-defined density. Surgical pathology: fibrocystic disease with intraductal epithelial proliferation and moderate atypia. *F*: Mammogram of a 66-year-old patient showing a mass 1.5 cm in diameter with smooth walls, "most likely represents a cyst or benign lesion such as fibroadenoma or papilloma; a well-circumscribed carcinoma cannot be totally excluded." Multihole needle aspiration revealed invasive ductal carcinoma of medullary type. *G*: Mammogram of a 61-year-old patient with Premarin-induced fibrocystic disease, showing a DY pattern. *H*: Sonogram of a 16-year-old patient showing a 5 by 6 cm hypoechoic mass with background echoes indicative of fibroadenoma.

recognized. In phase 1, fibrocystic changes are beginning in women in their mid-20s to late 20s to early 30s; cycles may be shortened (21 to 24 days) and premenstrual breast pain and/or tenderness of 1 week's duration may exist. Breast tissues show increased density

and tenderness. The upper outer quadrant is most frequently involved. In phase 2, there is progressive fibrocystic disease in patients in their 30s; nodularity (hard and tender areas of a few millimeters to 1 cm in diameter) and fibrocystic plaques and lumps (2 to 3 cm





**Fig. 11, I.** Surgical specimen (H) of a 5 by 6 cm fibroadenoma cut longitudinally.

in diameter) exist. At that phase, breast pain and tenderness are increased and extend to 2 to 3 weeks before menstruation or are permanent. In phase 3, there is advanced fibrocystic disease in women throughout their 40s. Breast pain and tenderness may extend for 2 to 3 weeks or be permanent and debilitating. Larger lumps and macrocysts (single or multiple) exist. Blue-domed cysts (1 to 3 cm in diameter) have a fibrous wall and contain a brown-greenish or ink-colored fluid (Fig. 10).

### Diagnosis of fibrocystic disease

**Signs and symptoms.** Fibrocystic disease has a history of many months to several years. Leading symptoms are breast pain (mastodynia) and/or tenderness; in 40% to 60% of patients these are associated with irregular menses, dysmenorrhea, menometrorrhagia, or ovarian cysts. Fibrocystic disease is rare in ovulating women, multiparous women, and patients using oral contraceptives. Breast pain is observed in the majority of patients. In more than half of the patients with mastoplasia (Table I), premenstrual breast swelling, mastodynia, and irregular menses are observed. Mastodynia may start a few days or 1 to 2 weeks before menstruation; it usually eases or subsides with the onset of or during menses. Under stimulation of ovarian sex steroids, the breast volume may increase up to 15% (edema, ductal-alveolar proliferation) toward the end of the menstrual cycle; similar stimulation also affects fibrocystic tissues. Because mammary edema and proliferative changes regress with a decrease in ovarian sex steroid production at the time of menstruation,<sup>2</sup> mastodynia eases or subsides with the onset of or during menses. However, in patients with progressed fibrocystic disease (pronounced nodularity, lumpiness, macrocysts), breast pain is often permanent. According to our experience, in 10% to 15% of patients little or no pain (high pain threshold?) is observed despite clinical and mammographic evidence of substantial fibrocystic disease. Also some patients accept premenstrual breast pain and tenderness as normal in the life of a woman and belittle it to their physicians or do not mention it at all.

**Table VI.** Management of fibrocystic disease and related abnormalities

#### *Fibrocystic disease*

Medroxyprogesterone acetate (10 mg/day) or norethindrone acetate (5 mg/day): days 15 to 25 of menstrual cycle (advanced disease: from day 10 to 25)

Low-estrogen oral contraceptives (Loestrin 1/20)

Vitamin E (400 to 1,200 IU/day) as adjunct therapy in patients with low serum levels of high-density lipoprotein

Breast mass: Multihole needle aspiration for tissue diagnosis

Epithelial atypia: Surgical excision

Macrocysts: Multihole needle aspiration for cyst fluid and tissue diagnosis

#### *Fibroadenoma (adenoma)*

Multihole needle aspiration for tissue diagnosis; surgical excision

#### *Duct ectasia (plasma cell mastitis)*

Multihole needle aspiration for tissue diagnosis and therapy (removal of abnormal tissues); surgical excision

#### *Papilloma*

Multihole needle aspiration for tissue diagnosis and therapy; surgical excision

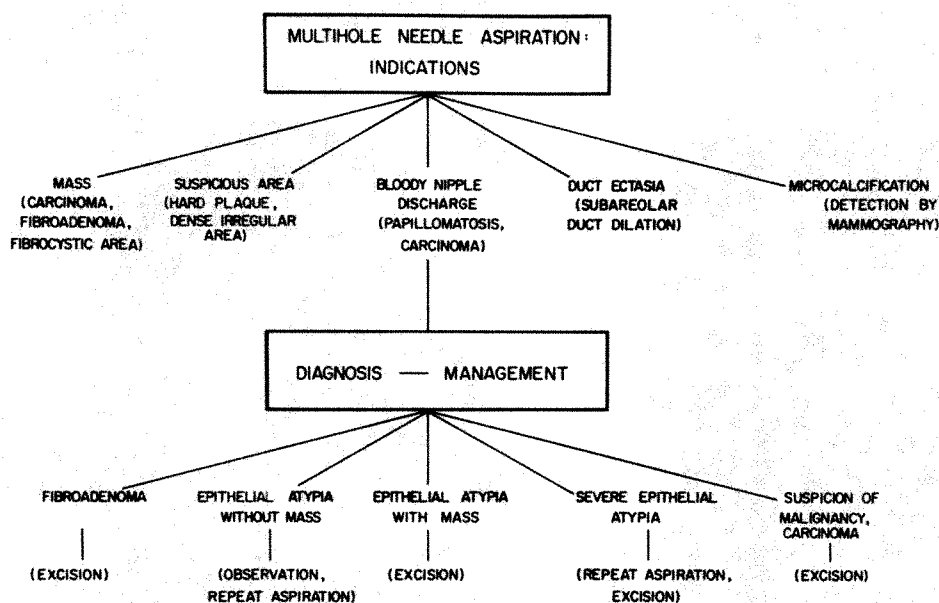
Breast pains are attributed to nerve irritation by edema of connective tissue and secretory retention (increase in pressure of dilated terminal ducts and cysts), as well as nerve pinching by processes of fibrosis and sclerosis. Also, an inflammatory round cell reaction to fibrocystic changes may produce neuralgia-like sensations due to nerve irritation and dolor owing to an inflammatory response and release of histamine by mast cells.

Pain unrelated to fibrocystic disease may be caused by (1) large, pendulous breasts (stretching of suspensory ligaments and tension on nerves); (2) intercostal neuralgia (spondylitis, obesity, respiratory infection, and cough); (3) Tietze syndrome, that is, painful and tender costochondral junction with pains projected to breast tissues; (4) biopsy scars (neuroma); (5) trauma (exacerbation of pain from fibrocystic disease and duct ectasia); and (6) psychoemotional disturbance. Therapy directed toward fibrocystic disease is usually not successful in these patients.

In approximately 20% of patients, axillary tenderness and enlarged lymph nodes are observed. Axillary lymph node swelling is in reaction to metabolic fibrocystic changes (breakdown product) and/or abacterial inflammatory round cell infiltration.

In contrast to a tender, irregular plaque or a lumplike fibrocystic tissue area or a papilloma, fibroadenoma is a nontender, circumscribed, freely movable mass with a smooth outline. The differential diagnosis of fibroadenoma entails a nontender cyst with a dense fibrotic wall and circular growing medullary and papillary carcinoma.<sup>12</sup> Usually cysts are tender, and very rarely an intracystic carcinoma (1 to 2 per 1000 carcinomas) co-





**Fig. 12.** Multihole needle aspiration for diagnosis and management of breast disease. In patients with isolated fibrocystic areas, intraductal proliferation (papillomatosis), microcalcification, and duct ectasia, multihole needle aspiration is diagnostic but can also be therapeutic because the abnormal tissue can be removed by aspiration. Fibroadenoma requires surgical excision. When the aspirate shows carcinoma, the patient is informed about the nature of the disease, and further diagnostic steps and treatment alternatives are discussed. (From Vorherr<sup>18</sup>.)

can feel and grossly appear like a fibrocystic lump but is usually not tender.

**Nipple secretion.** In one third of patients with fibrocystic disease, discharge is spontaneous or secretion can be expelled from the nipple. The cytologic features may include amorphous material (fat, proteins), ductal cells, erythrocytes, and/or foam cells (metabolic resorptive processes). The fluid is straw yellow, greenish, or bluish; unfortunately, such patients are often treated with antibiotics without success.

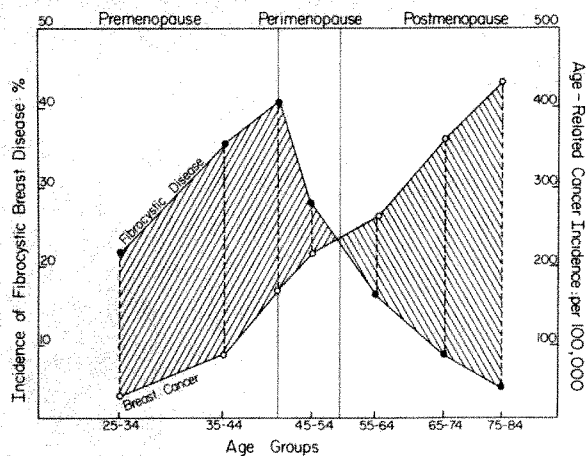
Patients with serosanguineous or bloody nipple secretion (pathologic secretion) require thorough investigation because carcinoma is found in one third (Table IV). The Papanicolaou-stained smear contains erythrocytes; foam cells, some containing hemosiderin (siderophages); normal ductal epithelial cells; atypical epithelium; and/or cancer cells.

**Mammography.** Mammographic evidence of fibrocystic disease is provided by a dense pattern (DY) (QDY: patients with DY pattern below age 35), as described by Wolfe.<sup>20</sup> Because of diffuse density (Fig. 11) it is difficult to discern underlying pathologic changes. A DY pattern is indicative of profuse connective tissue proliferation (fibrosis). Wolfe<sup>20</sup> observed a DY pattern in approximately 20% of women between age 39 and 49, in 5% between age 50 and 59, and in 0.5% of patients of age 60 or above. In patients with a DY pattern the risk of breast cancer is supposedly increased threefold to fivefold, but this has been disputed. Severe

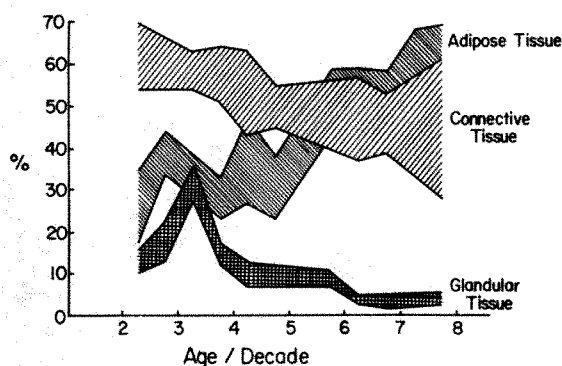
mammographic dysplasia has been associated with a fivefold to eightfold increased risk of breast cancer.<sup>21</sup> In our experience, a DY pattern is of diagnostic and prognostic value only when it is clinically correlated with fibrocystic disease, for the majority of premenopausal women without fibrocystic disease have a mammographic DY pattern. Also, almost 80% of carcinomas are diagnosed in postmenopausal women who have mainly N<sub>1</sub> (fatty breasts) and P<sub>1</sub> (minor ductal prominence) mammographic patterns, which are thought to be of minimal or low breast cancer risk.<sup>20</sup>

Epithelial changes in ducts and alveoli cannot be shown by mammography. However, presence of a pronounced dystrophic pattern and clinically progressed fibrocystic disease indicate that severe fibrous changes are most likely accompanied by intraductal epithelial proliferation. Beginning fibrocystic disease shows small dense areas, whereas epithelial-proliferative and nodular changes are reflected in the mammogram by darker specks amid dense white areas appearing as "buckshot" breast; microcalcifications may be present<sup>15</sup> (Table V). Calcification may be intraductal, intralobular, periductal-stromal, and intratumoral and is an unspecific tissue reaction in response to epithelial-proliferative processes with high metabolic activity, secretory retention, cellular damage, and necrosis.

In general, mammography is credited with an 80% accuracy of breast cancer diagnosis (Table V). In our experience with premenopausal patients with fibrocys-

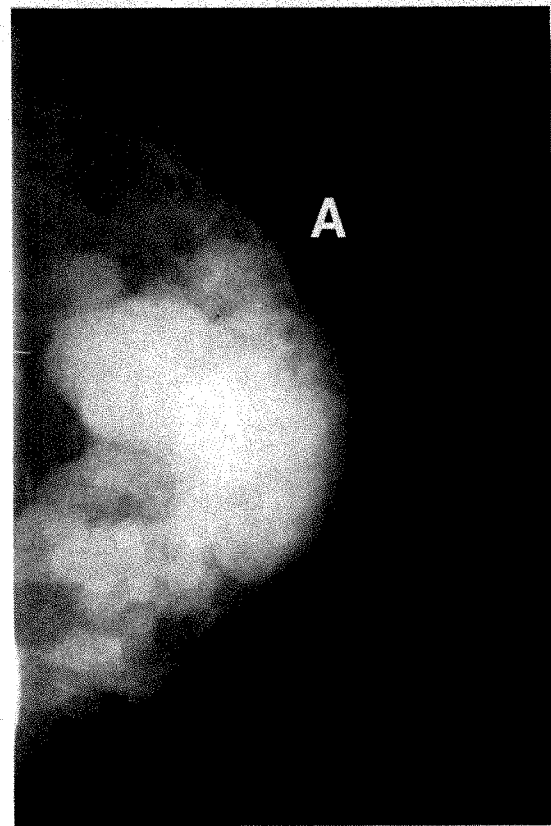


**Fig. 13.** Age-related incidence of fibrocystic breast disease and breast cancer. While the incidence of fibrocystic breast disease peaks at perimenopause and then steeply declines, the breast cancer incidence steadily increases with advancing age.



**Fig. 14.** Distribution of mammary tissues. In premenopausal women the amount of glandular tissue is four to five times that of postmenopausal women, in whom involuted ductal-alveolar structures are replaced by adipose tissue. (From Prechtel K. Altersabhängiger Strukturwandel der weiblichen Brustdrüse (Flächenprozentbestimmung). Verh Dtsch Ges Pathol 1970;54:393-7.)

tic disease (all with a dystrophic pattern on the mammogram), in 40% the cancerous lump was not diagnosed.<sup>22</sup> Nevertheless, in patients with fibrocystic disease beyond age 35, mammography is indicated. In patients before the age of 35, ultrasonography may be performed. For patients with a family history of breast cancer in first-degree relatives (mother, sister), a mammogram is advisable even before age 35, especially for patients with medium to large breasts. For patients above age 35 and with moderate to severe fibrocystic breast disease, regardless of other risk factors, a mammogram should be obtained. The mammography should be performed the week after menstruation (least tissue changes and edema). Mammography beyond age 40 should be repeated at 2-year intervals in patients without clinical symptoms and at 1-year intervals in

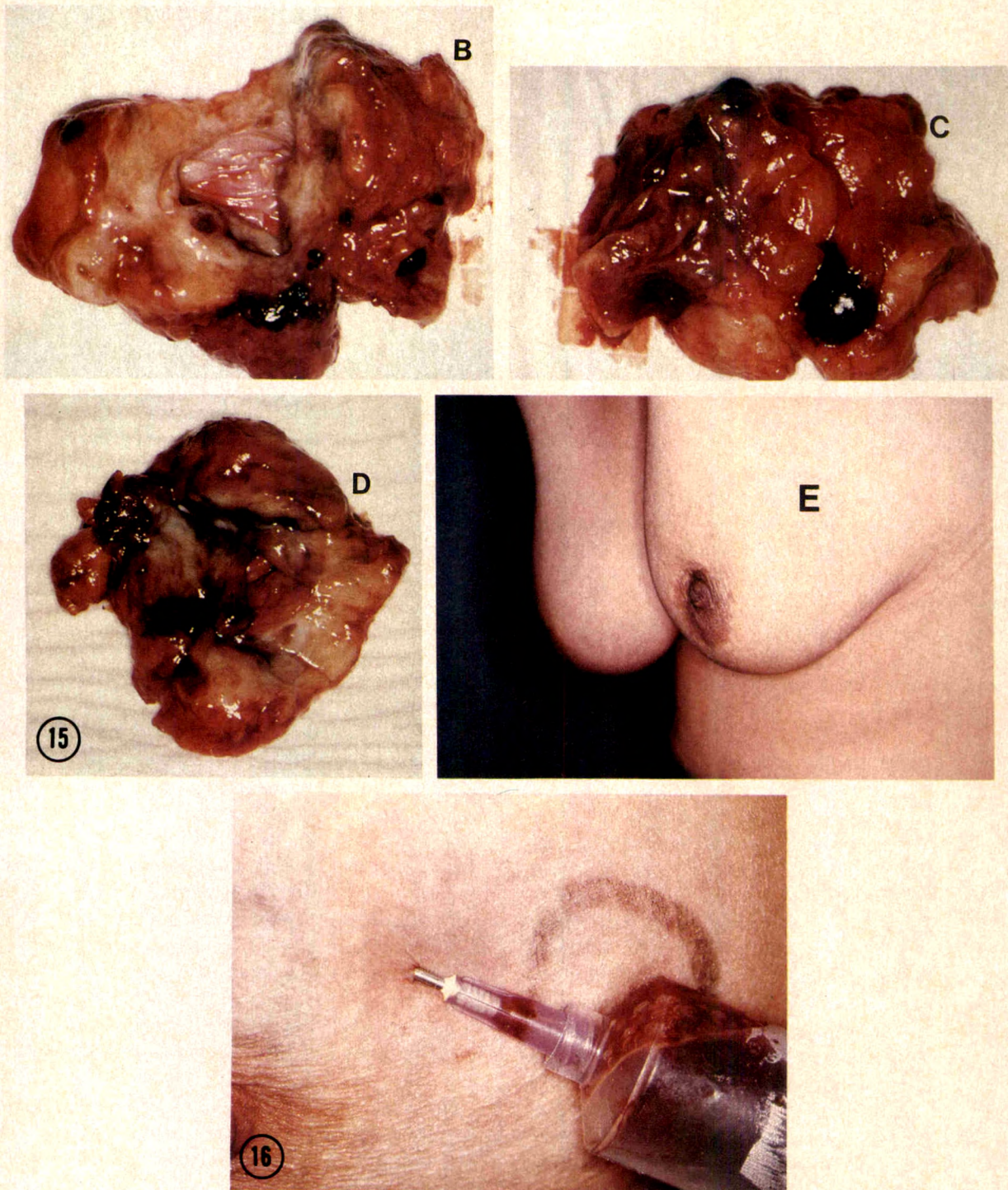


**Fig. 15, A.** Management of fibrocystic disease associated with epithelial atypia. Mammogram of a 42-year-old patient with advanced fibrocystic disease and breast lumpiness (macrocyts). Removal of 20 ml of greenish fluid by multihole needle aspiration from left breast. Aspiration histology: intraductal epithelial proliferation with moderate atypia.

patients at high risk. For women aged 50 and beyond, mammography should be done annually. According to present mammographic techniques the breast can probably tolerate 20 mammograms without the risk of radiation-induced cancer. In every patient with a suspicious mammogram and/or clinical findings, multihole needle aspiration biopsy is indicated (Fig. 12).

**Sonography.** Sonography (ultrasound) is a diagnostic technique that complements x-ray mammography. In patients with fibrocystic disease, mammary echography by ultrasound reveals echo-dense, inhomogeneous nodular-fibrous areas and anechoic masses, that is, cysts (Fig. 11, B). Mammary echoes depend on tissue elasticity and the various interphases of tissue components. Homogeneous tissues such as fibroadenoma, despite predominance of fibrous tissue, are hypoechoic with some echoes inside the mass and lack of definition of a posterior wall (Fig. 11, H). Small cysts and enlarged ducts can be visualized as anechoic areas with a defined posterior wall. In this respect, the diagnostic value of ultrasound is superior to that of mammography. On





**Fig. 15, B to E.** Management of fibrocystic disease associated with epithelial atypia. Surgical specimens of patient in A. Fibrocystic lumps from left breast (*B* = 3 by 5 cm, *C* = 4 by 5 cm, *D* = 5 by 5 cm in diameter) revealing blue-domed cysts, intraductal epithelial proliferation with slight to moderate atypia. *E*: Patient's left breast 3 months after removal of three large fibrocystic lumps (*B*, *C*, *D*).

**Fig. 16.** Multihole needle aspiration in a 64-year-old patient with painful areas of lumpy duct ectasia. Removal of 15 ml of viscous yellowish material for tissue diagnosis and therapy.



**Table VII.** Loestrin treatment of 182 patients with fibrocystic breast disease

Evaluation	Response to treatment		
	First treatment cycle	Third treatment cycle	Sixth treatment cycle
Patient self-evaluation			
Relief of breast pain and/or tenderness	81% (Moderate)	86% (Moderate)	93% (Good)
Clinical examination			
Tenderness	92% (Moderate)	95% (Moderate)	90% (Good)
Nodularity, lumpiness	26% (Minor)	62% (Minor)	88% (Moderate)

Side effects include: breakthrough bleeding, 23%; weight gain, 4%; weight loss 3%; increase in libido, 12%; decrease in libido, 8%; nervousness, irritability, or depression, 4%.

the mammogram a solitary cyst with pronounced pericystic fibrosis may not differ from a fibroadenoma or a well-circumscribed carcinoma.<sup>13</sup> Perfection of ultrasonic techniques may result in better documentation not only of fibrocystic disease but also, and perhaps to even a greater degree, of malignant changes. At present, sonography is most useful for follow-up of the patient with fibrocystic breast disease and as an aid to discovery of carcinoma in radiologically dense (DY) breasts (Table V).

**Needle aspiration biopsy.** Needle aspiration biopsy is indicated in patients with a breast mass (cyst), a lump-like structure, a hard dense area, or any other abnormal tissue area, as defined by clinical examination, mammography, or sonography (Fig. 12, Table V). In patients at high risk of breast cancer, needle aspiration is performed when the slightest suspicion arises.<sup>17, 18</sup> In women with fibrocystic disease, ductal epithelium consists of cohesive cells with a scant rim of cytoplasm and round or oval small, slightly hyperchromatic nuclei; apocrine metaplasia may be present (Fig. 4). Connective (fibrous) tissue is usually predominant. Numerous epithelial strands in a tubular-lobular arrangement are typical of fibroadenoma; the nuclei are slightly enlarged (8 to 10  $\mu$ m in diameter), vary in size, and are moderately hyperchromatic (Fig. 9). Papilloma is indicated by polygonal, larger cells with light-colored, normal nuclei and a clear rim of cytoplasm (Fig. 7). Epithelial atypia is defined by a moderate increase and variation in nuclear size, unclear nuclear outline, pleomorphism, and mild hyperchromatism (Fig. 8). Cancer cells reveal an increase in nuclear size, pronounced nuclear pleomorphism and hyperchromatism (chromatin clumping), an increased rate of mitosis, a loss in cellular cohesion, and enhanced visibility of nucleoli.

#### Clinical relevance of fibrocystic disease, duct ectasia, and intraductal proliferation in postmenopausal women

After menopause, glandular epithelium and fibrocystic tissue changes involute. However, breast invo-

lution may be accompanied and followed by abnormal changes such as intraductal epithelial proliferation and duct ectasia, which may create as much of a problem as fibrocystic disease in premenopausal patients.

**Fibrocystic disease.** After menopause, the ovaries continue to secrete small amounts of estrogens for another 3 to 5 years. After the age of 55, ovarian estrogen secretion is negligible and fibrocystic disease declines sharply while the breast cancer incidence steadily increases (Fig. 13). With decrease and cessation of ovarian estrogen secretion, mammary lobular-alveolar structures involute and are replaced by adipose and connective tissue; only larger ducts remain<sup>2</sup> (Fig. 14). Also fibrocystic changes involute.

Even though fibrocystic changes are observed at the time of autopsy in 25% of postmenopausal women aged 60 to 80,<sup>1</sup> only about 10% of cases are clinically symptomatic. In these patients, fibrocystic breast disease is usually iatrogenic, that is, the consequence of estrogen replacement therapy with inappropriately high doses. Trapido et al.<sup>23</sup> observed a significant increase in fibrocystic disease with estrogen replacement therapy. Also, in some postmenopausal women (especially obese patients), estrogens derive from peripheral conversion of ovarian and adrenocortical androstenedione to estrone in adipose tissue, liver, muscle, and tissues of reproductive organs in amounts that may induce or maintain fibrocystic disease.

Fibroadenoma in a postmenopausal woman is extremely rare and persists from premenopause; such fibroadenomas are hard, involuted, and may be calcified.<sup>12</sup>

**Duct ectasia and intraductal proliferation.** Clinically, in 40% to 80% of postmenopausal patients, duct ectasia of up to several centimeters in diameter with pains due to secretory retention is diagnosed; in approximately 15%, axillary lymph node swelling exists.<sup>1</sup> Because of pain, swelling, and redness, the disorder may mimic infectious mastitis. Rupture of such a dilated duct may lead to periductal chemical irritation and abacterial inflammation with appearance of round cells



**Table VIII.** Oral contraceptives and breast cancer risk

<i>Series</i>	<i>Duration</i>	<i>Relative risk</i>	<i>Comment</i>
Centers for Disease Control <sup>32</sup> (1983)	Ever use	0.9	Neither duration of use nor time since first use alters risk of breast cancer
	≥11 yr	0.8	
Centers for Disease Control <sup>33</sup> (1984)	4-6 yr	0.8	
	>6 yr	0.9	No association between high-progestin pills and breast cancer
Pike et al. <sup>34</sup> (1983)	4-6 yr (use before age 25)	2.0	"High"-progestin pill use for 2-4 yr and for 4-6 yr increases breast cancer risk by 2.4-fold and 4.1-fold, respectively
	> 6 yr	4.9	
Vessey et al. <sup>35</sup> (1983)	Ever use, age 16-35	0.9	No patterns of breast cancer risk emerge in younger women
	Interval since last use:		
	1-4 yr	0.9	
	4-8 yr	1.2	
	>8 yr	0.5	
Hennekens et al. <sup>36</sup> (1984)	Ever use	1.0	More than 10 yr users have slightly lower breast cancer risk than never-users
Rosenberg et al. <sup>37</sup> (1984)	Ever use	1.0	Use of oral contraceptives for ≥5 yr was not associated with breast cancer
	≥5 yr	1.0	

(plasma cells, lymphocytes, histiocytes). This involves processes of phagocytosis and development of granulation tissue that contains gray, red, or yellow (xanthomatous) areas<sup>1,24</sup> (Fig. 5). This so-called plasma cell mastitis, which is a nonpurulent process, may present as an acute, subacute, chronic, or recurrent acute inflammatory and very painful tumorlike (2 to 4 cm in diameter) lesion. Secondary bacterial infection may exacerbate inflammation and result in abscess formation with skin perforation and persistent pus secretion. Also, granuloma formation (granulomatous mastitis) with leukocytes, epithelioid cells, giant cells, and a peripheral lymphocyte wall may develop.<sup>24</sup> The differential diagnosis of carcinoma is made on the basis that malignant neoplasia is usually hard and irregular but not tender.

The clinical course of duct ectasia encompasses three phases. Phase 1 consists of formation and retention of secretory material. Phase 2 is characterized by ductal rupture and development of acute, abacterial plasma cell mastitis (fever, chill, hyperemia, tumorlike infiltrate: complicated duct ectasia). Phase 3 includes persistence of subacute and chronic intraductal and periductal granulating and sclerosing mastitis (tumorlike infiltrate: plasma cells, lymphocytes, histiocytes).

Duct ectasia may be followed by duct stenosis, obliteration, and/or scar formation with periductal and intraductal hyalinosis and elastosis causing skin or nipple retraction; it also may be associated with intraductal epithelial-papillary (lumplike) proliferation (Fig. 5, *H*). According to Golinger,<sup>4</sup> intraductal hyperplasia was observed in 69% of patients older than 70 years.

#### Medical treatment of fibrocystic disease

Before institution of therapy, the patient requires a thorough clinical workup and documentation of the

extent of fibrocystic disease; carcinoma must be ruled out (Fig. 10). In some patients breast discomfort is not related to fibrocystic disease and hormonal therapy is without benefit.

Patients with breast pain and tenderness should be advised to wear light, loose clothing and a well-padded brassiere.

Medical treatment is provided with the intent to stop progression of fibrocystic disease and to relieve breast pain and tenderness. The long-term goal is to reverse fibrocystic changes and soften breast tissues. Therapeutic response is controlled in 3- to 4-month intervals. Relief of breast pain and/or tenderness is documented. By clinical examination tissue density, tenderness, degree of nodularity, and lumpiness are evaluated. When indicated, sonography and/or mammography are used as further aids in judging the treatment response.

Diuretics, thyroid hormones, progestogens (progesterone and progesterone derivatives), progestins (19-nortestosterone derivatives), androgens (danazol), antiestrogens (tamoxifen), antiprolactin (bromocriptine), vitamins (A, B<sub>1</sub>, B<sub>6</sub>, and E), dihydroergotamine, and abstinence from methylxanthines (tea, coffee, chocolate) have been used in the treatment of fibrocystic disease.

Therapy with diuretics, iodine-containing agents, thyroid hormones, vitamin A, and vitamin B has not proved successful. Also, thyroid hormone therapy cannot be recommended as treatment. We have observed hypothyroid patients who developed fibrocystic disease on a regimen of thyroid medication.

**Antiprolactin treatment.** With the assumption of a role of prolactin in the development of fibrocystic breast disease, antiprolactin treatment with bromocriptine (Parlodel) has been applied and supposedly has a 60% to 80% success rate. However, prolactin is most

likely not involved in the pathophysiology of fibrocystic disease and breast cancer; rather, it may be protective.<sup>9</sup> In most patients with fibrocystic disease plasma prolactin levels are normal. Therefore, prolactin depression is not rational. Since many patients desire birth control, administration of bromocriptine, which increases fertility and the chance of undesirable pregnancy, is contraindicated. We have patients in whom fibrocystic disease developed or progressed while they were on a regimen of long-term antiprolactin treatment for infertility and pituitary microadenoma. Bromocriptine exerts side effects such as nausea, vomiting, edema, hypotension, dizziness, hair loss, peptic ulcer, headache, and hallucinations in 50% to 60% of patients; a substantial proportion (~20%) discontinue treatment. Also, bromocriptine has been found teratogenic in rabbits.<sup>25</sup> At this time, physicians are very reluctant to prescribe bromocriptine for the treatment of fibrocystic breast disease, particularly since the long-term sequelae are unknown.

**Vitamin E.** The therapeutic basis of vitamin E for the treatment of fibrocystic breast disease is its function as an antioxidant. Vitamin E inhibits oxidation of essential cellular constituents (radical-induced peroxidation) and thereby protects from formation of toxic biochemical products. Accordingly, vitamin E regulates the synthesis of specific proteins and enzymes required in differentiation and adaptation of tissues.

Vitamin E (600 IU/day) has been reported to be beneficial ("objective and subjective remission") in 85% of patients with fibrocystic breast disease.<sup>26</sup> London's group<sup>27</sup> also reported that vitamin E benefited patients with premenstrual syndrome, the mechanism of beneficial action being unknown. However, London et al.<sup>28</sup> subsequently contradicted their former findings.

Vitamin E may reduce the risk of atherosclerosis and cardiovascular disease by increasing high-density lipoproteins and decreasing low-density lipoproteins.<sup>29</sup> In contrast, progestins as contained in oral contraceptives cause an undesirable increase in low-density lipoproteins and a decrease in high-density lipoproteins. Therefore, we add natural-source vitamin E (400 to 1200 IU/day) for patients receiving Loestrin or norethindrone acetate or medroxyprogesterone acetate who are 40 years of age or older and/or in whom serum levels of high-density lipoproteins or low-density lipoproteins are borderline or abnormal (Table VI). In women with a family history of breast cancer or who are obese or have diabetes mellitus or breast cancer, high-density lipoproteins were found to be low.<sup>9</sup> Therefore, we encourage vitamin E intake especially as an adjunct to hormonal treatment, because it has no side effects and it can only be beneficial.

**Oral contraceptives.** Patients on a regimen of combination oral contraceptives are protected from devel-

opment of fibrocystic disease and related pathologic conditions such as adenoma, fibroadenoma, and atypical hyperplasia.<sup>30</sup> For treatment of fibrocystic disease with oral contraceptives success rates of 70% to 90% have been reported.<sup>31</sup> In some women breast tissues are very estrogen-sensitive. Thus oral contraceptives that contain 0.035 or 0.05 mg of ethinyl estradiol may induce mastodynia and fibrocystic changes, especially when their progestin potency is relatively low. Accordingly, we use a preparation (Loestrin 1/20) with a low estrogen (0.02 mg of ethinyl estradiol) and a relatively high progestin content, that is, 1 mg of norethindrone acetate, which is twice as potent as norethindrone without the acetyl group (Table VI). The treatment rationale for use of this pill is to reduce ovarian estrogen secretion, while the small estrogen amount of Loestrin is modulated in breast tissues by its progestin component. In our series of 182 patients with fibrocystic disease, Loestrin 1/20 provided relief of subjective symptoms (pain, tenderness) and clinical success in approximately 90% of patients within 3 to 6 months (Table VII). In 80% of patients breast pain is alleviated after the first pill cycle. Clinical improvement of fibrocystic changes could be documented in 88% of patients after 6 months of treatment. In approximately 15% of patients, Loestrin 1/20 does not initially provide or sustain adequate ovarian suppression; this is evidenced by recurrence of normal or increased menstrual flow (not light as usual) and/or cramping and by persistence of mastodynia. In these patients, norethindrone acetate (2.5 mg/day) or medroxyprogesterone acetate (5 mg/day) for 21 days is added to the pill regimen. This is successful in most patients. Also, changing to Loestrin 1.5/30 is usually of benefit. Because the development of fibrocystic disease takes many months or several years, treatment (and contraception) is provided for at least 1 to 2 years. Unfortunately, mastodynia recurs in 30% to 40% of patients upon discontinuance of treatment.

Side effects of Loestrin treatment are mainly intermenstrual spotting and bleeding, which occur in 23% of patients during the first 3 to 4 months of pill intake. Thereafter, usually no significant intermenstrual bleeding is observed.

It is generally accepted that use of oral contraceptives does not increase the risk of breast cancer<sup>32</sup> (Table VIII). When the progestin content of the oral contraceptive is high, the risk of breast cancer seems reduced.<sup>30</sup>

**Progestogens and progestins.** The therapeutic basis for application of progestogens (progesterone or progesterone derivatives) and progestins (19-nortestosterone derivatives) is suppression of pituitary-ovarian function and opposition of the effect of estrogen on breast tissues. Use of progestogens or progestins during

the luteal phase of the cycle has resulted in improvement of fibrocystic disease in approximately 80% of patients.<sup>31</sup>

A success rate of 85% was obtained in our series of 176 patients. Breast pain and tenderness are alleviated in 70% of patients during the first treatment cycle, that is, 10 mg of medroxyprogesterone acetate from day 15 to day 25 of the menstrual cycle. Breast tenderness and fullness may remain the same or become aggravated during the first treatment cycle in 30% and such patients need reassurance. After the second or third cycle, breast pain and tenderness are greatly alleviated or disappear entirely in almost all women. Three months is required in 80% of patients to document clinical success of treatment, that is, disappearance of tenderness on palpation; and at least 6 months is needed to observe regression of fibrocystic changes (nodularity) and softening of breast tissues. We have obtained similar results with cyclic use of progesterone (50 mg) suppositories.

Treatment with medroxyprogesterone acetate (10 mg/day) from day 4 to day 25 of the cycle is instituted in patients with pronounced fibrocystic disease and epithelial proliferation or those who do not respond to cyclic therapy between day 15 and day 25. Whereas treatment from day 4 to day 25 is very successful, intermenstrual spotting or bleeding observed in all patients requires the addition of 0.02 to 0.04 mg of ethinyl estradiol per day. We now treat these patients with medroxyprogesterone acetate (10 mg) from day 10 to day 25 of the cycle, and intermenstrual bleeding is less likely to occur. Since the progestogen is given early in the menstrual cycle, ovarian estrogen secretion is depressed and the synergistic effect of the progestogen with endogenous estrogens, resulting in bloating and breast fullness, is circumvented. Spotting during the last days of medication may occur. Because 10% to 20% of patients on a regimen of medroxyprogesterone acetate experience mild to moderate depression, we change the medication in these patients to norethindrone acetate (5 mg/day) or add 0.02 to 0.04 mg of ethinyl estradiol to the daily progestogen regimen. Reducing the progestogen dose to 5 mg/day and adding a small dose of 50 mg of danazol per day is also helpful. Depression is usually limited to the first two or three progestogen treatment cycles.

A desirable side effect is the diuretic action of medroxyprogesterone acetate, which antagonizes the effect of aldosterone on the distal nephron. Patients on a regimen of progestogen observe relief of premenstrual swelling of ankles, hands, and breasts and notice more frequent voiding of urine. Patients who are nervous and worrying types feel relaxed and comfortable with medroxyprogesterone acetate. The progestogen is also beneficial for women with rough skin and hirsutism, making the skin smoother and counteracting facial hair growth.

Progestogen treatment is usually provided for 9 to 12 months; then the drug is discontinued and the patient is followed up in 3- to 4-month intervals. Approximately 40% of the patients eventually require reinstitution of treatment because of recurrence of breast pain and tenderness.

**Danazol.** Danazol, an androgen derivative (17 $\alpha$ -nortestosterone), has been found beneficial in 70% to 90% of patients treated. The dose regimen is 100 to 600 mg/day for 2 to 6 consecutive months.<sup>38-40</sup> In 1980, Nezhat et al.<sup>41</sup> reported that 200 and 400 mg of danazol per day for 6 months brought about elimination of breast nodularity in 47% and in 75% of patients, respectively. We have never observed complete elimination of breast nodularity with danazol, or any other treatment, within 6 months. The therapeutic basis for danazol is its effect on the hypothalamus, pituitary, and ovary, resulting in suppression of ovarian function. Danazol decreases follicle-stimulating hormone and luteinizing hormone secretion (negative hypothalamic feedback) and blocks the effect of these hormones on ovarian tissues. Amenorrhea develops in all patients treated with 400 to 600 mg of danazol per day.<sup>39</sup> Even with 200 mg of danazol per day, more than 50% of patients become amenorrheic within 4 months.

In contrast to earlier reports, according to which danazol produced few or no side effects,<sup>38</sup> recent studies indicate that this drug has substantial adverse effects. In 60% to 70% of patients these include such symptoms as oily skin, acne, hirsutism, deepening of the voice, sweating, hot flashes, atrophic vaginitis, muscular spasms, hypertension, liver dysfunction (cholestatic jaundice), headaches, and edema. Moreover, amenorrhea is not acceptable to premenopausal women, many of whom are reluctant to take danazol. Since the long-term consequences of danazol are as yet undetermined and because treatment results obtained with low-estrogen oral contraceptives and with progestogen are equal or superior to those obtained with danazol, we have abandoned the sole use of this drug.

### **Surgical treatment of fibrocystic breast disease and related pathologic conditions**

Ultrasound, mammography, needle aspiration, and/or surgical excision are performed in patients with fibrocystic disease and a suspicious area, as determined by clinical examination (Fig. 12). Multihole needle aspiration for cytologic and histologic examination<sup>18</sup> can spare 80% of patients surgical biopsy. Generally, in 30% of patients with fibrocystic disease, intraductal epithelial proliferation is diagnosed. We observe epithelial proliferation in 90% of patients who undergo surgical biopsy after multihole needle aspiration.

Fibrocystic disease is diagnosed in approximately 60% of all surgical breast biopsy specimens, cancer in 25%, and fibroadenoma in 10%.

Although, the risk of malignancy for patients with fibroadenoma is less than 1%, surgical excision is recommended. Most patients with a fibroadenoma will request that the lump be removed. Also, a surgical cosmetic problem may arise when a fibroadenoma is allowed to grow too big.

Cysts of  $\geq 1$  cm in diameter require aspiration for cytologic and histologic examination.<sup>18</sup> Suspicion for intracystic carcinoma is aroused when the aspirate contains atypical or cancer-suspicious epithelial cells. In such cases, surgical excision of the area of the cyst is indicated. With our multihole needle aspiration technique,<sup>17,18</sup> in which fluid and 5 to 10 ml of material are removed from the cystic and pericystic areas, we usually do not observe refilling of cysts.

For patients with an extremely high risk of breast cancer (breast cancer in mother and sister) and/or suspicious findings (severe atypical epithelial proliferation) or women with therapy-resistant fibrocystic disease and severe breast pain, subcutaneous mastectomy with an implant or bilateral reduction mastectomy is recommended.<sup>12</sup> However, almost all high-risk patients reject this option and request close follow-up. In these patients we perform selected and/or random multihole needle aspiration for both cytologic and histologic examination<sup>18</sup> and, when indicated, surgical excision. Even several larger fibrocystic lumps can be removed without breast deformation (Fig. 15).

#### Treatment of postmenopausal women

Fibrocystic changes in postmenopausal patients are usually the consequence of estrogen replacement therapy. Therefore, estrogen administration is greatly reduced and treatment with a progestogen is instituted. For the first 2 to 4 weeks, 10 mg of medroxyprogesterone acetate is given daily, then 5 mg of the progestogen is administered daily for 2 to 4 weeks. Thereafter, every second or third day 5 mg of the progestogen is given, possibly with 0.02 mg of ethinyl estradiol. Most women can be "weaned" from estrogen and do fine with progestogen alone, with pain and breast tenderness subsiding. Patients with preceding breast cancer, at high risk of breast cancer, or with duct ectasia are treated with a progestogen only. For patients with pruritus vulvae, a dry vagina, and vulvar-vaginal atrophy accompanied by dyspareunia, we advise local application of a lubricant and eventually estrogen cream in small amounts. Progestogen therapy opposes mammary intraductal epithelial proliferation, relieves hot flashes and sweating, and facilitates intestinal absorption of calcium and its retention in bone to counteract osteoporosis. Progestogen therapy is also successful in patients with painful duct ectasia.

Multihole needle aspiration is performed whenever

suspicion of carcinoma arises. Patients with painful, lumpy areas of duct ectasia, complicated by periductal plasma cell mastitis, often require removal of inflamed tissues by multihole needle aspiration (Figs. 5 and 16). Surgical excision is rarely necessary.

#### Fibrocystic disease—A nondisease?

Fibrocystic breast disease has been labeled a "nondisease."<sup>43</sup> According to Love,<sup>44</sup> the term fibrocystic disease is "misleading and frightening" and "harmless fibrocystic disease is a blanket term meaning little more than lumpy breasts without cancer." However, it is generally accepted that fibrocystic disease is a pathophysiologic entity which increases the risk of breast cancer (Table II). Also, fibrocystic disease can be incapacitating and thus impair the quality of life. Patients with fibrocystic disease may have pronounced mastodynia, which makes it impossible for them to lie on their stomach or touch their breasts. When treatment of fibrocystic disease is neglected and the disease progresses, breast lumpiness becomes a predominant feature. Lumps consist of fibrocystic tissue, macrocysts with pericystic fibrosis, duct ectasia, and sclerosing adenosis.

The grave danger in considering fibrocystic disease a harmless nondisease is that such labeling might keep women with breast lumps and nodularity from seeking diagnosis and treatment.<sup>22</sup> Feeling that they have nothing to worry about, they might fail to seek clarification as to whether the lumps or nodularity may be progressing fibrocystic disease or cancer. A woman who does seek clarification and who is told that she has "harmless" nonproliferative lumpiness may find false reassurance in this diagnosis, for she might actually have epithelial atypia (Fig. 6) or even a carcinoma. In these patients, invasive diagnostic procedures such as needle aspiration and surgical biopsy become necessary.

Within the last 2 years we have diagnosed breast cancer in 18 patients (age range 29 to 48 years) with fibrocystic disease. All had a dystrophic (DY) pattern in the mammogram. In six of these cancer patients the "harmless" lump did not show in the mammogram; in two other patients, radiographically and sonographically the lump indicated "harmless" fibrocystic disease. In another patient, the mammogram and sonogram suggested that the lump was a fibroadenoma. In view of the clinical problems with fibrocystic disease and the increased risk of carcinoma, no clinician who cares for patients suffering from fibrocystic disease would call this a "nondisease." Because of the limited value of mammography and sonography for the diagnosis of breast cancer in patients with fibrocystic disease, we always perform multihole needle aspiration when the slightest suspicion arises.

Women with fibrocystic changes and clinical complaints need to be thoroughly diagnosed and managed. Breast surgical procedures can be prevented in many



patients if fibrocystic disease, instead of being labeled "harmless," is recognized as the threat it is and adequately treated in time.

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# Infective endocarditis in obstetric and gynecologic practice

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Infective endocarditis is an important but uncommon complication in obstetric or gynecologic practice; we found only 124 cases reported in English and selected European papers during the last 40 years. The majority of cases (74%) were caused by streptococci; viridans streptococci predominated, while enterococci and group B streptococci were uncommon except after abortion. The overall mortality rate was 29%, while the mortality rate for the fetus when the mother developed infective endocarditis was 23%. The incidence of endocarditis in this setting is low and seems to be decreasing. Therefore, the risk-benefit ratio may not favor routine use of prophylaxis for endocarditis. We conclude that antibiotics need not be given for prevention of endocarditis before most common obstetric and gynecologic procedures. These include uncomplicated vaginal deliveries, uncomplicated spontaneous or induced abortions, dilatation and curettage, insertion or removal of intrauterine contraceptive devices (in the absence of pelvic infection), and biopsies of the cervix. For patients in whom both the underlying heart lesion and the obstetric or gynecologic procedure seem to pose significant risk for endocarditis, prophylaxis should be given. Two parenteral regimens for patients at highest risk are recommended: ampicillin plus gentamicin or vancomycin plus gentamicin. For lower-risk situations, one oral regimen is suggested: amoxicillin. (AM J OBSTET GYNECOL 1986;154:180-8.)

**Key words:** Prophylaxis, infective endocarditis, endocarditis, antibiotics, pregnancy

The literature dealing with infective endocarditis in obstetric and gynecologic patients consists mainly of scattered case reports. Cases of endocarditis have been reported during pregnancy or associated with intrauterine devices, abortion, vaginal delivery, or cesarean section, but the relative risks associated with each of these situations is unknown. The efficacy of prophylactic antibiotics is unproved, and firm guidelines for the prevention and treatment of endocarditis in obstetric and gynecologic patients are lacking. Certain high-risk patients can be identified, but the necessity for prophylaxis in the majority of women at lower risk needs to be reevaluated. Administration of antibiotics during pregnancy and lactation is associated with special risks to both the mother and infant; both therapeutic and prophylactic antibiotic regimens must be tailored to these considerations. We have reviewed the literature on infective endocarditis related to obstetric and gynecologic practice, in order to evaluate some of these problems and to formulate reasoned approaches for use in practice.

## Sources

The period chosen for review extends from the beginning of the antibiotic era, in the early 1940s, to the present. A computer-assisted search was used to obtain

the literature on cases of endocarditis related to obstetric or gynecologic events from 1966 through 1983. References published between 1940 and 1966 that were cited in these articles were reviewed in order to find other cases. The criteria for inclusion of cases in our review were: (1) a clinical picture consistent with infective endocarditis plus positive blood cultures or (2) endocarditis diagnosed at autopsy. Cases from the preantibiotic era were deliberately excluded. This was done to allow estimates of mortality that would be more meaningful today. Our review was based primarily on the English literature, but several articles in French and German were included.

## Results

Our search for reports of infective endocarditis associated with obstetric or gynecologic events yielded only 99 cases from the English literature that met the straightforward entry criteria (Table I). Most of these occurred in association with pregnancy (49 cases)<sup>1-36</sup> and the puerperium (18 cases),<sup>3, 17, 20, 37-46</sup> which together accounted for 68% of the total. We found only 25 reported cases following abortions<sup>7, 8, 37, 39, 40, 45, 47-52</sup>; these were most often related to pelvic infections associated with criminal abortion. Only three cases have been noted in women with intrauterine contraceptive devices<sup>45, 53, 54</sup> and three after routine dilatation and curettage.<sup>8, 40, 55</sup> One case occurred after hysterectomy.<sup>56</sup>

Cases of endocarditis were reported more commonly during the earlier years covered by this review: 54 cases between 1940 and 1960 and only 41 between 1960 and

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Table I. Precipitating obstetric or gynecologic event in relation to etiologic organisms

Event	Viridans streptococci	Group B streptococci	Enterococci	Other streptococci	S. aureus	Other*	Organism not stated
Pregnancy	23	1†	3†	6	8†	9	1
Puerperium	4	1	3	5	2	2	1
Abortion							
Spontaneous			1	2	1		
Therapeutic	1	2	3				
Illegal	1		1	5			
Type not stated		2	4		2		
Dilatation and curettage			2			1	
Intrauterine contraceptive device	1	1		1			
Hysterectomy						1	
Total	30	7	17	19	13	13	2

\**N. gonorrhoeae*, two; *Pseudomonas*, two; *S. enteritidis*, one; *L. monocytogenes*, one; *H. aphrophilus*, one; *C. trachomatis*, one; *N. catarrhalis*, one; *S. epidermidis*, one; *M. polymorpha*, one; *C. hominis*, one.

†Polymicrobial infection with *S. aureus* plus group B streptococci in one patient and *S. aureus* plus enterococcus in another. Three patients had culture-negative endocarditis.

Table II. Frequency of underlying heart disease and outcome in cases with known causes

Organism	No.	%	Underlying heart disease		Maternal outcome (survival)	
			No./total	%	No./total	%
Streptococci						
Viridans	30	74	25/28	89	25/28	89
Group B*	7		2/7	29	6/7	86
Enterococci*	17		10/11	91	5/8	63
Unclassified	19		12/16	75	11/15	73
<i>Staph. aureus</i> *	13	13	5/11	45	7/12	58
Others†	13	13	9/13	69	5/12	42
Total	99	100	63/85	74	57/80	71

\*Polymicrobial infection with *S. aureus* plus group B streptococci in one patient and *S. aureus* plus enterococcus in another. Three patients had culture-negative endocarditis.

†*S. enteritidis*, one; *L. monocytogenes*, one; *H. aphrophilus*, one; *N. gonorrhoeae*, two; *C. trachomatis*, one; *N. catarrhalis*, one; *Pseudomonas*, two; *S. epidermidis*, one; *M. polymorpha*, one; *C. hominis*, one.

1983. Rheumatic or congenital heart disease was listed as the most common predisposing condition in patients in whom a case history was provided. Only 21 reports of endocarditis on normal heart valves were found; in four cases the organism was *Staphylococcus aureus*,<sup>4, 26, 40</sup> in four cases group B streptococci,<sup>37, 14, 51</sup> and in three cases viridans streptococci.<sup>2, 13, 40</sup> Other species of streptococci were noted four times<sup>24, 42, 50, 52</sup> and unusual organisms in four instances.<sup>25, 28, 36, 47</sup> One patient had polymicrobial infection with both *S. aureus* and group B streptococci,<sup>27</sup> and another was infected with *S. aureus* and enterococcus.<sup>35</sup>

The spectrum of etiologic organisms was similar to that found in endocarditis in most other patient groups. Viridans streptococci were most frequent (30 cases<sup>1-13, 17, 19, 20, 22, 31, 34, 38, 40, 43, 45, 46, 54, 55</sup>) followed by enterococci (17 cases<sup>35, 38, 43</sup>), and *S. aureus* (13 cases<sup>20, 21, 26, 27, 32, 35, 37, 40</sup>). Various other species of streptococci not identified as viridans, enterococci, or group B streptococci caused 19 cases<sup>3, 14, 20, 24, 27, 33, 37, 38, 40, 42, 48, 50, 52</sup>

or 19% of the total. It is likely that this sizable group of "other species of streptococci" actually included some viridans streptococci and some enterococci. In spite of the fact that group B streptococci are frequently present in the genitourinary system in women, these organisms caused only seven cases.<sup>27, 37, 44, 51, 53</sup> Two cases were caused by *Pseudomonas*,<sup>40, 41</sup> and two by *Neisseria gonorrhoeae*.<sup>16</sup> One case each was caused by *Listeria monocytogenes*,<sup>25</sup> *Salmonella enteritidis*,<sup>29</sup> *Hemophilus aphrophilus*,<sup>23</sup> *Chlamydia trachomatis*,<sup>28</sup> *Neisseria catarrhalis*,<sup>56</sup> *Mima polymorpha*,<sup>18</sup> *S. epidermidis*,<sup>15</sup> and *Cardiobacterium hominis*.<sup>56</sup>

Underlying cardiac disease was present in 63 (75%) of the 84 patients for whom this information was provided (Table II). Overall survival was 71%. Only 50% of patients who acquired the disease in the puerperium survived, even though the cause and year of onset did not differ significantly from those of other groups in our study. (A specific statement regarding survival or death was given for only 14 of the 18 cases occurring

in the puerperium.) The total number of cases associated with use of an intrauterine contraceptive device, hysterectomy, or dilatation and curettage was too small to allow calculation of meaningful mortality rates.

Cases of endocarditis in obstetric and gynecologic practice were reported more commonly during the first 20 years of the period reviewed. Fifty-four (57%) of the 94 cases in which a year of onset was given occurred before 1960. After 1960, we could find only 25 reports of cases occurring during pregnancy<sup>12-16, 18, 19, 21, 23-26</sup> and four during the puerperium.<sup>42-45</sup> No cases after dilatation and curettage have been reported since the 1950s. Endocarditis after abortion has also been reported less frequently in the last two decades. Seventeen cases were reported before 1960 and only eight<sup>15, 47-52</sup> after that year. Not surprisingly, all of the three cases associated with an intrauterine contraceptive device<sup>45, 53, 54</sup> have been reported in the last 12 years.

**European experience.** A survey of articles from France and Germany indicated some differences from the experience reported in the English literature. Review of nine articles from the French literature revealed 25 cases of endocarditis reported since 1960.<sup>57-65</sup> Underlying cardiac diseases were not specified for most of these patients, but one had had chorea as a child.<sup>65</sup> A high proportion of these cases (56%) occurred after illegal or septic abortions.<sup>57, 58, 60, 62-65</sup> *S. aureus* was the predominant organism, causing 15 of 25 cases (60%).<sup>57, 58, 62-64</sup> Gram-negative organisms were recovered in five instances,<sup>61</sup> group B streptococci in three,<sup>59, 65</sup> and enterococci in two,<sup>60, 61</sup> one of which also had *Streptococcus bovis*<sup>60</sup> bacteremia. In contrast, tricuspid valve involvement was much more frequent than in the English language series, occurring in 88% of these cases.<sup>57-60, 62, 64, 65</sup> Endocarditis followed a primary septicemia in at least 11 cases<sup>61</sup> and after local infection progressing to septic thrombophlebitis in two instances.<sup>62</sup> Fourteen cases were confirmed at operation or at necropsy.<sup>58-60, 62, 64</sup> Tricuspid valve involvement occurred in only nine (9%)<sup>13, 21, 35, 42, 44, 47, 51, 52</sup> of the 99 cases we collected from the English literature, and primary septicemia diagnosed before the apparent onset of endocarditis was uncommon. Overall mortality in these European cases was 20%, a rate similar to that reported in the English literature for patients with tricuspid valve infection.

Two cases of endocarditis in association with pregnancy were found in the German literature,<sup>66, 67</sup> both developing spontaneously in the third trimester of pregnancy. One of these involved the tricuspid valve<sup>67</sup> and was confirmed at operation. Neither patient was reported to have had underlying cardiac disease.

**Endocarditis during pregnancy (excluding the puerperium).** Forty-nine cases of endocarditis occurred during pregnancy.<sup>1-36</sup> The disease was diagnosed in the

first trimester in four cases, in the second trimester in 16, and in the third trimester in 21 instances. The time of onset was not reported in eight cases. No obvious explanation emerged for the striking increase in incidence after the first trimester.

Viridans streptococci accounted for 23 cases (47%).<sup>1-6, 9-13, 17, 22, 31, 34</sup> Only three cases were caused by enterococci<sup>7, 8, 35</sup> and one by group B streptococci.<sup>27</sup> *S. aureus* was cultured simultaneously with the group B streptococci in that case and also from one of the cases infected with enterococci.<sup>35</sup> The remaining 22 cases were due to other species of streptococci (six cases<sup>14, 20, 24, 33</sup>), *S. aureus* (six cases<sup>20, 21, 26, 32, 35</sup>), and one case each caused by *N. gonorrhoeae*,<sup>16</sup> *L. monocytogenes*,<sup>25</sup> *S. enteritidis*,<sup>29</sup> *H. aphrophilus*,<sup>23</sup> *M. polymorpha*,<sup>18</sup> *S. epidermidis*,<sup>15</sup> *C. trachomatis*,<sup>23</sup> and *C. hominis*.<sup>36</sup> In two cases no organism was identified.

Endocarditis occurred on an abnormal cardiac valve in 32 (74%) of the 43 cases in which the patient's previous cardiac status was mentioned. As in other settings, viridans streptococci were especially likely to infect abnormal valves; this occurred in 20 (91%) of 22 instances in which sufficient information existed to determine underlying diseases in relation to the infecting organism.

The likely portal of entry was identified in 15 instances (31%).<sup>2, 3, 5, 10, 12, 17, 20, 24, 27, 33, 35</sup> Dental procedures preceded endocarditis in nine cases; in all nine the organisms were streptococci (five viridans streptococci<sup>3, 5, 10, 12, 17</sup>), one group D streptococcus,<sup>24</sup> and three unspecified streptococcal species.<sup>20, 33</sup> One patient had pneumonia<sup>20</sup> and another had a sore throat<sup>2</sup> prior to onset of endocarditis. Four patients were intravenous drug abusers and in all four the infecting organism was *S. aureus*.<sup>27, 35</sup> In two cases of polymicrobial infection, *S. aureus* was isolated along with another organism: group B streptococci<sup>27</sup> and *Streptococcus fecalis*.<sup>35</sup>

The prognosis for mothers-to-be with endocarditis was fair. In 36 of 47 cases (77%) the patients survived. Two patients with *S. aureus* and two with viridans streptococcal infection died, as did one with enterococcus, four of the eight with unusual organisms, and one of the two with no organism identified. As has been noted by other authors,<sup>17, 22, 68</sup> pregnancy itself does not seem to influence the course of endocarditis for the worse. Fetal survival was also fairly good; 30 of 39 (77%) fetuses survived. In one case an elective abortion was performed at 20 weeks,<sup>1</sup> while in another spontaneous abortion occurred at 28 weeks.<sup>3</sup> Two infants died 2 days after their mothers underwent emergency valve replacement,<sup>17, 35</sup> and another died after premature labor at 7 months.<sup>27</sup> No cause was stated for the death of one infant.<sup>30</sup> Two maternal deaths occurring in the first trimester and one at 30 weeks accounted for the remaining three fetal deaths.<sup>18, 19</sup>



**Endocarditis during the puerperium.** Bacterial endocarditis followed vaginal delivery in 16 instances<sup>3, 17, 20, 37-42, 44, 45, 54</sup> and cesarean section in only two.<sup>17, 55</sup> As with the other cases in this series, most occurred between 1940 and 1960. Fourteen (74%) were reported between 1940 and 1960, and only four after 1960. Both cases of endocarditis after cesarean section occurred in the 1940s.

Of the 17 cases in which organisms were identified, 13 (56%) were caused by streptococci. Of these, four were caused by viridans streptococci,<sup>17, 40, 45, 54</sup> three by enterococci,<sup>20, 38, 55</sup> one by group B streptococci,<sup>44</sup> and five by other streptococcal species.<sup>3, 37, 39, 42</sup> *S. aureus* accounted for two cases<sup>40</sup>; *Pseudomonas* endocarditis occurred twice. One of these patients had the onset of premature labor at 7 months with a prolonged third stage.<sup>41</sup> The other was hospitalized 2 days for severe preeclampsia before delivery and suffered a postpartum hemorrhage.<sup>40</sup> Both women died.

Underlying cardiac disease was identified in 14 of 17 (82%) evaluable cases. As in the cases reported during pregnancy, viridans streptococci, enterococci, and other streptococci usually infected patients with preexisting valvular heart disease. The only case of group B streptococcal endocarditis occurred in a patient with no known heart disease.

Potential predisposing factors associated with development of postpartum endocarditis included premature labor, prolonged rupture of the membranes before delivery, a prolonged third stage of labor, and manual removal of the placenta. One of our patients had premature labor,<sup>41</sup> one had internal version, one had manual removal of the placenta,<sup>40</sup> and another had a prolonged third stage with the placenta removed by external uterine massage.<sup>41</sup> In two cases postpartum hemorrhage was noted.<sup>40</sup> One woman who was delivered at home with the use of forceps developed enterococcal endocarditis. Another apparently uneventful home delivery also was followed by development of enterococcal endocarditis.<sup>38</sup> Underlying pelvic infection was present in two instances, and in both *S. aureus* was cultured.<sup>40</sup> An unusual case developed following a lumbar extradural block in which a sterile extradural fluid collection was noted at operation and group B streptococci were cultured from the blood.<sup>44</sup>

Maternal mortality was high (50%) when endocarditis developed in the puerperium. There was no obvious explanation for this observation. One contributing factor was that two of these cases were caused by *Pseudomonas*, an organism that is difficult to treat.

One case of *Streptococcus mutans* endocarditis occurred despite administration of prophylaxis with cephalosporin at the time of delivery.<sup>54</sup> The patient had underlying rheumatic mitral insufficiency and the organism was resistant to cephalosporins.

**Endocarditis after abortion.** Endocarditis following abortion is uncommon. Of 25 reported cases,<sup>7, 8, 37, 39, 40, 45-52</sup> four followed spontaneous abortion,<sup>7, 37, 39, 48</sup> six followed therapeutic abortion,<sup>45, 46, 49, 51</sup> seven resulted from criminal abortion,<sup>7, 39, 40, 50, 52</sup> and in eight cases the type of abortion was not stated.

The incidence of infective endocarditis in this setting is not known precisely. In the United States 1,034,200 abortions were performed in 1975 alone,<sup>69</sup> but only three associated cases of endocarditis were reported during the decade 1970 through 1980. Thus, even allowing for underreporting, the incidence of endocarditis must be well below 1 per 1 million abortions.

Seventeen cases were reported between 1940 and 1960, but only eight cases in the next 23 years. Thus the incidence of endocarditis after abortion seems to have decreased. This is likely due at least in part to the decrease in the proportion of criminal abortions and the infectious complications that often followed.

The predominant organisms in endocarditis after abortion were enterococci (nine cases, 36%<sup>7, 8, 46, 49</sup>), group B streptococci (four cases, 16%<sup>37, 51</sup>), and other streptococcal species (seven cases, 28%<sup>39, 40, 48, 50, 52</sup>). Viridans streptococci were responsible for only two cases<sup>40, 45</sup> and *S. aureus* for three.<sup>37, 47</sup> Only in this subgroup of patients did group B streptococci and enterococci assume the importance that might be expected of them after obstetric and gynecologic events. Again it is likely that some of the "other streptococcal species" were actually enterococci.

In 14 of 20 cases (72%) there was underlying cardiac disease. Group B streptococci infected normal valves in three of four cases.<sup>37, 51</sup> Enterococcus occurred in patients with abnormal hearts in five instances where cardiac status was reported.<sup>7, 46, 49</sup> Anaerobic streptococcal endocarditis occurred in one patient with no underlying heart disease.<sup>52</sup>

Overall survival was 63% (10 of 16 cases for which outcome was reported) for patients with endocarditis after abortion. Survival was approximately the same in each subgroup and was not related in these cases to type of organism. All but one of the reported deaths occurred in cases with onset prior to 1955.

#### **Endocarditis in other obstetric and gynecologic settings**

**Intrauterine contraceptive devices.** Only three cases of endocarditis complicating use of an intrauterine contraceptive device have been reported.<sup>45, 53, 54</sup> Group B streptococci, viridans streptococci, and *N. gonorrhoeae* were each recovered in one patient. All patients had known underlying cardiac disease. Two cases were associated with overt pelvic infection<sup>53, 54</sup> and in the third the organism was *N. gonorrhoeae*.<sup>45</sup> Yellow vaginal discharge and expulsion of the device occurred 1 week after insertion in one patient who developed endocarditis.<sup>53</sup>

Despite the large number of women using intrauterine contraceptive devices and the significant rate of associated pelvic infection (2.5% in the year after insertion<sup>70</sup>) intrauterine contraceptive devices rarely cause infective endocarditis. The rate, while not known precisely, must be less than 1 per 1 million patient years.

**Dilatation and curettage.** Endocarditis followed dilatation and curettage in three cases, all of which occurred prior to 1960. Enterococci were cultured in two instances<sup>55</sup> and a nonhemolytic streptococcus in the other.<sup>40</sup> One patient reportedly underwent dilatation and curettage for vaginal discharge<sup>40</sup>; in the others no mention was made of accompanying pelvic infection before or after the procedure.

Two of the three cases of endocarditis associated with dilatation and curettage occurred in patients with rheumatic valvular disease; in the other, cardiac status was not reported. Likewise, in only two cases was maternal outcome reported; both these women survived.

**Hysterectomy.** We were able to find only one case of endocarditis complicating hysterectomy.<sup>56</sup> This case occurred in a 45-year-old woman with a normal heart. Hysterectomy had been undertaken for severe menometrorrhagia; the organism was *N. catarrhalis*.

### Comment

Our review indicates that the incidence of infective endocarditis after obstetric and gynecologic procedures is low and falling. The incidence calculated by other authors for all deliveries varies from 0.03 to 0.14 per 1000.<sup>17,20</sup> In the subgroup with preexisting cardiac disease, the rate has been calculated to be from 5.5 to 9 per 1000.<sup>6,22</sup> Because infective endocarditis is not a notifiable disease, these incidence rates cannot be regarded as accurate. The true incidence today is probably much lower. Factors that presumably have contributed to this favorable trend are: (1) a decreasing incidence of chronic rheumatic heart disease, which was the underlying lesion in 75% of cases in the 1940s, (2) decreased incidence and improved management of obstetric and gynecologic infections in the antibiotic era, and (3) legalization of abortion, thereby decreasing the proportion of criminal abortions. Use of antibiotic prophylaxis in patients with preexisting cardiac disease during obstetric and gynecologic events that could cause bacteremias may have contributed, but this is probably a minor factor compared with the three listed above.

The fact that iatrogenic procedures can cause transient bacteremias that sometimes result in infective endocarditis, usually on previously damaged cardiac valves, has been recognized since the 1930s.<sup>71-73</sup> Blood cultures are positive immediately after dental procedures in 30% to 80% of patients<sup>71-73</sup> and more than 200

cases of bacterial endocarditis after dental and urologic procedures have been reported.<sup>74,75</sup> Enterococcus is the likely etiologic organism in cases of endocarditis associated with urologic procedures.<sup>75-77</sup>

Whereas the frequency and characteristics of bacteremias after dental procedures have been well studied,<sup>71,72</sup> there have been very few studies of bacteremias after obstetric and gynecologic events. Positive blood cultures occur in only 1.0% to 4.9% of patients after uncomplicated vaginal delivery.<sup>78-81</sup> This figure is very low when compared with the high frequency of positive blood cultures immediately after dental procedures.<sup>71,72</sup> We could find no evaluation of the incidence of bacteremia in patients delivered by cesarean section. Everett et al.<sup>70</sup> found no significant bacteremia after intrauterine contraceptive device insertion (84 patients) or removal (16 patients). Likewise, Regetz et al.<sup>82</sup> found no significant bacteremias after punch biopsy of the cervix in 40 patients. Livengood et al.<sup>83</sup> recently reported two isolates of streptococci from blood samples taken immediately after endometrial brushing and biopsy in 50 patients. One of these was *S. fecalis*, while the other unfortunately was not speciated. Also isolated were three other vaginal organisms that do not pose a significant risk for endocarditis and two probable contaminants. These data<sup>32,83</sup> are consistent with the conclusion that bacteremias do occur after biopsy but with very low frequency. The incidence of transient bacteremia after abortion, dilatation and curettage, or hysterectomy or in patients having intrauterine contraceptive devices in place and pelvic inflammatory disease has not been systematically evaluated.

The use of prophylactic antibiotics to prevent infective endocarditis is widely accepted as part of standard medical practice.<sup>71,73,84</sup> Because no controlled clinical trials have been done to prove the efficacy of prophylaxis in man, this practice is based on theoretical considerations. Current empiric recommendations<sup>84-86</sup> have taken into account secondary sources of information such as case reports,<sup>87</sup> antibiotic susceptibilities, and results of experimental studies in vitro and in animals.<sup>88-91</sup>

Present standards of care require that prophylactic antibiotics should be used when any iatrogenic procedure that is anticipated to cause bacteremia with significant frequency is performed in a patient with a congenital or acquired heart lesion that poses a significant risk for endocarditis.<sup>71,84,91</sup> Obviously, the interpretation of "significant" in this context is subject to debate. While recognizing that the data are scanty, we conclude that the rates of bacteremia to be expected after many common obstetric and gynecologic events (such as normal delivery<sup>78-81</sup> and biopsies<sup>82,83</sup>) are too low to justify routine use of antibiotic prophylaxis. This should be reserved for selected, higher risk situations, such as

dilatation and curettage in the presence of active infection. Our conservative conclusion is supported by the rarity of reported cases of endocarditis in these settings, especially after diagnostic procedures.

Certain cardiac lesions appear to carry a relatively high risk: these include prosthetic valves, ventricular septal defects, asymmetric septal hypertrophy, aortic stenosis, patent ductus arteriosus, cyanotic congenital heart disease, acquired rheumatic valvular disease, and previous infective endocarditis.<sup>91</sup> Mitral valve prolapse presents a special problem. This is a very common abnormality, affecting approximately 5% of the population, including many young women who will experience the usual obstetric and gynecologic events. Although mitral valve prolapse cannot be regarded as a high-risk lesion, it increases an individual's risk for endocarditis by a factor of fivefold to eightfold.<sup>92</sup> Many authors currently recommend prophylaxis for patients with mitral valve prolapse, especially those with a systolic murmur consistent with mitral insufficiency. However, decision analysis<sup>93</sup> indicates that attempted prophylaxis for dental procedures might not be cost-effective, because the incidence of endocarditis in this setting is low. In fact, use of parenteral prophylaxis might cause a net loss of life: the risk of death from anaphylaxis might exceed the risk from endocarditis.<sup>93</sup> These considerations can probably be extrapolated to many obstetric or gynecologic procedures, which seem to pose an even lower risk than dental work. Although the analysis<sup>93</sup> cited above has not been confirmed by any clinical trial, it might be prudent to use oral rather than parenteral prophylaxis whenever practicable for patients with mitral valve prolapse during obstetric and gynecologic procedures. This is because the risk of death from endocarditis in this setting may be even lower than the very low risk of death from penicillin-induced anaphylaxis.

To conclude, the decision on whether to give antibiotics for attempted prophylaxis of endocarditis in obstetric or gynecologic settings must be individualized. Two risk factors should be assessed: the underlying cardiac lesion and the procedure that might cause bacteremia. If *both* seem to pose significant risks, for example, in prolonged labor after premature rupture of the membranes in a patient with a prosthetic valve, prophylaxis for endocarditis should be given. If *only one* poses significant risk, for example, normal delivery in a patient with a prosthetic valve, use of prophylaxis should be considered optional. If *neither* is associated with significant risk, for example, uncomplicated insertion or removal of an intrauterine contraceptive device in a patient with mitral valve prolapse, prophylaxis should be omitted. Suggested regimens are listed in Table III.

Whether or not prophylaxis was used, any patient

**Table III.** Authors' current recommendations for prophylaxis of endocarditis in patients at risk, during obstetric and gynecologic procedures\*

Standard parenteral regimen	Ampicillin, 2.0 gm intramuscularly or intravenously, plus gentamicin, 1.5 mg/kg intramuscularly or intravenously ½ to 1 hr before
Alternative parenteral regimen for penicillin-allergic patients	Vancomycin, 1.0 gm intravenously slowly during 1 hr, starting 1 hr before, plus gentamicin, 1.5 mg/kg intramuscularly or intravenously
Oral regimen for minor genitourinary tract procedures†	Amoxicillin, 3.0 gm orally 1 hr before, then 1.5 gm 6 hr later

Note: (1) These regimens are empiric suggestions; no regimen has been proved effective for prevention of endocarditis and prevention failures may occur with any regimen. (2) These regimens are not intended to cover all clinical situations; the practitioner should use his own judgment on safety and cost-benefit issues in each individual case. (3) One or two additional doses may be given if the period of risk for bacteremia is prolonged.

†No ideal oral regimen for penicillin-allergic patients is known. Clindamycin, 600 mg orally 1 hour before, then 300 mg orally 6 hours later, may be tried.

with a known cardiac lesion should report symptoms consistent with endocarditis promptly, and the physician should be alert to the possibility after an obstetric or gynecologic event.

**Safety of antibiotics in pregnancy.** The choice of antibiotics for prevention or treatment of endocarditis in pregnancy should take both mother and fetus into consideration. The number of antibiotics actually shown to be safe for use in pregnancy is small. The penicillins, which all cross the placenta, are generally regarded as safe for both the mother and the fetus. Likewise, the cephalosporins and erythromycin are considered to be safe enough for use in pregnancy if necessary.<sup>94, 95</sup> Chloramphenicol is toxic for the neonate but does not pose any greater risks for the pregnant women or fetus than for other patients.<sup>94</sup> Aminoglycosides can cross the placenta in small quantities and cause fetal ototoxicity.<sup>95, 96</sup> Sulfonamides can be used during the first and second trimesters in pregnant women; although they do not harm the fetus in utero, they may cause kernicterus in neonates and therefore should be avoided in the third trimester.<sup>95, 97</sup> Co-trimoxazole should be avoided in early pregnancy because of its antifolate effects and the possible risk of congenital abnormalities.<sup>94</sup> Its use in late pregnancy is contraindicated because one of its components is a sulfonamide. Tetracyclines are contraindicated throughout

pregnancy; they can cause severe, even fatal, hepatic necrosis in pregnant women. Moreover, tetracyclines given from midpregnancy onward can deposit in fetal bones and teeth, causing unsightly staining.<sup>95, 98</sup>

Antibiotics for prevention or treatment of infective endocarditis in the pregnant woman must be chosen with the preceding considerations in mind. Fortunately, most regimens include one of the  $\beta$ -lactam antibiotics, which are generally regarded as safe. When an aminoglycoside must be included, as in treatment of enterococcal endocarditis, the duration of use should be as short as possible. Today, gentamicin is probably preferable to the more traditional streptomycin because serum concentrations can be measured more easily and most physicians are more familiar with its use. Important considerations in guiding aminoglycoside dosage are the greater volume of distribution in pregnant women and the increased renal clearance.<sup>99, 100</sup>

**Antibiotics for nursing mothers.** Most antibiotics administered to nursing mothers will enter breast milk.<sup>95, 101</sup> Concentrations in milk are usually rather low; in most instances these will neither provide a therapeutic effect in the infant nor cause significant toxicity. If a nursing mother were given high-dose therapy for a prolonged period, especially if any degree of renal failure were present, higher concentrations could appear in milk. This, combined with the immature hepatic and renal function of infants, could cause delayed inactivation and/or delayed excretion, leading to significant concentrations of antibiotics in the infant's serum. Sulfonamides are an important exception; even in the low quantities found in breast milk these drugs can cause kernicterus in neonates or hemolytic anemia in infants who are deficient in glucose-6-phosphate dehydrogenase.<sup>95, 101</sup>

For most nursing mothers with endocarditis the safest approach would be to discontinue nursing during antibiotic therapy because of the high concentrations of drugs usually used and the long duration of treatment. This would be especially advisable in cases requiring therapy with antimicrobial agents other than  $\beta$ -lactam antibiotics.

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# Effect of ritodrine on uteroplacental blood flow and cardiac output distribution in unanesthetized pregnant guinea pigs

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The effects of ritodrine,  $15 \mu\text{g} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$  infused intravenously for 2 hours, on cardiac output distribution and uteroplacental blood flow were investigated in 10 chronically catheterized guinea pigs between 57 and 63 days of pregnancy. Ritodrine produced an average 29% increase in cardiac output, three quarters of which was distributed to the carcass and myocardium. Absolute placental blood flow decreased by 10%, and the placental fraction of cardiac output decreased by one third. Uteroplacental vascular resistance increased by 41% during ritodrine infusion. The proportional changes in placental blood flow were positively correlated and those in placental vascular resistance negatively correlated with the change in cardiac output. (AM J OBSTET GYNECOL 1986;154:189-94.)

**Key words:** Uteroplacental blood flow, cardiac output, regional blood flows, ritodrine, guinea pigs

$\beta$ -Adrenergic agonists are widely used in clinical obstetrics for the inhibition of premature labor. Some studies with indirect methods of blood flow measurement have suggested an increase in uteroplacental flow during administration of these drugs to pregnant women, especially in cases where diminished blood flow was suspected on the basis of the clinical findings.<sup>1-5</sup> These results have not been confirmed by studies with direct methods of flow measurement in sheep.<sup>6-8</sup> In guinea pigs Martensson et al.<sup>9</sup> observed an increase in placental blood flow during infusion of ritodrine,  $12 \mu\text{g} \cdot \text{min}^{-1}$ , but a decrease with  $120 \mu\text{g} \cdot \text{min}^{-1}$ .

Use of the sheep as a model for the study of human uteroplacental blood flow, especially vasodilatation, has been criticized by Bell,<sup>10</sup> who instead proposed use of the guinea pig. The guinea pig studies of Martensson et al.<sup>9</sup> were, however, acute observations carried out under anesthesia; it is possible that the experimental conditions might have affected their results. We present here our observations of the effects of ritodrine on the circulation of pregnant guinea pigs, obtained with use

of a chronic preparation to avoid the stress of operation and with microspheres to measure changes in the distribution of cardiac output as well as regional blood flow distribution within the uterus.

### Material and methods

Ten pregnant guinea pigs with known durations of pregnancy were used in this study. Polyethylene catheters (inside diameter of 0.40 mm and outside diameter of 0.80 mm, or inside diameter of 0.58 mm and outside diameter of 0.96 mm) were inserted into the left ventricle via the left common carotid artery, into the abdominal aorta via the femoral artery, and into the right external jugular vein. The operations were carried out under general anesthesia (ketamine hydrochloride,  $45 \text{ mg} \cdot \text{kg}^{-1}$  subcutaneously and xylazine hydrochloride,  $4 \text{ mg} \cdot \text{kg}^{-1}$  intramuscularly) between the forty-fourth and fifty-second days of pregnancy. All catheters were tunneled subcutaneously to the interscapular region and there exteriorized. The catheters were filled with heparinized saline solution ( $25 \text{ units} \cdot \text{ml}^{-1}$ ), and were flushed every second day. The animals were allowed to recover for 7 to 15 days. Their conditions were assessed from food intake and weight gain.

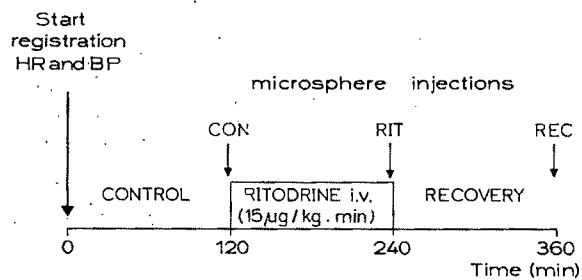
The experimental observations were carried out between the fifty-seventh and sixty-third days of pregnancy (mean, 59.6 days) with the animals unrestrained in the same cages in which they had been housed since before implantation of the catheters. The plan of the experiments is shown in Fig. 1. The first microsphere

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**Fig. 1.** Diagram of the plan of the experiments. The timing of microsphere injections is indicated by the short arrows. *HR*, Heart rate; *BP*, blood pressure; *CON*, control; *RIT*, ritodrine; *REC*, recovery.

injection was carried out after a control period of 120 minutes. Ritodrine was then infused via the jugular vein catheter at a rate of  $15 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}$  for 120 minutes. This dose was chosen on the basis of a pilot study to give a sustained increase of 10% to 15% in maternal heart rate. The volume infused was  $1 \text{ ml} \cdot \text{hr}$ . The second microsphere injection was carried out at the end of the infusion period and the third injection after a 2-hour recovery time. The maternal aortic blood pressure was recorded continuously during the experiment (except during the collection of the reference samples), and the maternal heart rate was counted from the blood pressure tracing.

Radionuclide-labeled microspheres ( $15 \mu\text{m}$ ) obtained from New England Nuclear, Inc., were prepared by suspension in 0.9% saline solution with 5% dextran and 0.01% Tween 80. Two combinations of labels were used: cerium 141, ruthenium 103, and scandium 46 (four animals); and cobalt 57, tin 113, and scandium 46 (six animals). Before injection the suspension was heated to  $38^\circ \text{C}$  and stirred continuously. Each injectant contained approximately 105 microspheres per kilogram estimated maternal weight, diluted to a volume of 1 ml. The microspheres were injected over 60 seconds by hand via the left ventricular catheter, and the catheter was then flushed with 1 ml of saline solution. The reference sample was withdrawn from the aortic catheter at a rate of  $0.6 \text{ ml} \cdot \text{min}$ , from 30 seconds before the start of the injection until 90 seconds after the end of the flush.

About 5 minutes after the third microsphere injection the animal was killed by means of an overdose of pentobarbital. The position of the catheters was checked at autopsy, and the aortic and jugular venous catheters were found to be correctly placed in all cases. In only six animals, however, was the left ventricular catheter found to be properly positioned. The tip of the catheter was just distal to the aortic valve in three animals, and in one other the catheter lay in the descending thoracic aorta. Organs were dissected and prepared for counting as described by Peeters et al.<sup>11</sup>

Counting was done with a Packard Autogamma Scintillation Counter Model 5220. The radioactivity from each label was calculated after correction for background and for spillover from the other labels. This latter was calculated from standard samples of each label. Organ flows were calculated as follows:

$$\text{flow (organ)} = \frac{\text{flow (reference)} \times \text{counts (organ)}}{\text{counts (reference)}}$$

Cardiac output was taken as the sum of the organ flows. In the three animals in which the ventricular catheter had prolapsed into the ascending aorta, the myocardium contained a disproportionately high number of microspheres. Therefore, the mean myocardial blood flow of the six animals with correctly placed catheters was used in calculating the cardiac output of these three animals. In the animal in which the injection catheter lay in the descending aorta, only the flows in organs and tissues below the diaphragm were determined, and no cardiac output was calculated. Equal distribution of counts in the two kidneys indicated that mixing had been adequate in this case.

Mean arterial blood pressure was determined from the pulsatile pressure according to the following formula:  $(\text{systolic pressure} + 2 \text{ diastolic pressure}) / 3$ . Total peripheral resistance per 100 gm of body weight was calculated from the mean arterial pressure divided by cardiac output (milliliters per minute per 100 gm). Uteroplacental vascular resistance was calculated from the mean arterial blood pressure divided by placental blood flow (milliliters per minute per 100 gm), thus excluding myometrial blood flow.

Results are presented as means and standard errors. Differences between values were evaluated statistically with use of the Wilcoxon matched-pairs signed-rank test. Correlations were assessed by means of the Spearman rank test. Significance levels are for two-tailed tests.

## Results

After losing weight during the 2 to 4 days following the operation, the animals gained weight at an average rate of  $13 \text{ gm} \cdot \text{day}^{-1}$ . Maternal weight at the time of the experiments ranged from 718 to 1225 gm (mean,  $949 \pm 48 \text{ gm}$ ). The average litter size was 3.3, with a range from two to five. Seven of the 10 animals carried only live fetuses, while the other three each had one live fetus and together seven dead fetuses. Only the results from the live fetuses ( $n = 26$ ) were used in the calculations of mean fetal weight ( $61 \pm 3 \text{ gm}$ ), mean litter weight ( $160 \pm 27 \text{ gm}$ ) and placental blood flow.

Infusion of ritodrine,  $15 \mu\text{g} \cdot \text{min}^{-1} \cdot \text{kg}$ , increased maternal heart rate and cardiac output significantly (Table I). Mean arterial blood pressure changed minimally, and total peripheral resistance decreased. All



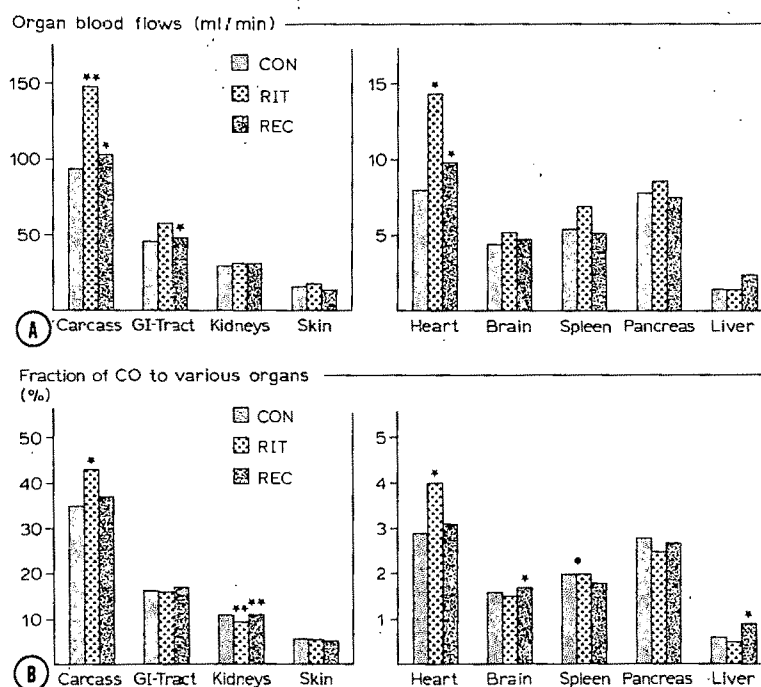


Fig. 2. Changes in organ blood flows (A) and fractions of cardiac output distributed to various organs (B) during and after infusion of ritodrine in pregnant guinea pigs. \* =  $p < 0.05$  and \*\* =  $p < 0.01$  compared to preceding values. CON, Control; RIT, ritodrine; REC, recovery.

Table I. Cardiovascular values (mean  $\pm$  SE) before infusion, after 2 hours of infusion of ritodrine ( $15 \mu\text{g} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ ), and after a 2-hour recovery period in guinea pigs in late pregnancy

	Control*	Ritodrine	Recovery
Heart rate (beats $\cdot$ min $^{-1}$ )	255 $\pm$ 5	284 $\pm$ 5†	260 $\pm$ 6
Stroke volume (ml)	1.07 $\pm$ 0.05	1.24 $\pm$ 0.09	1.06 $\pm$ 0.07‡
Cardiac output (ml $\cdot$ min $^{-1}$ )	271 $\pm$ 11	350 $\pm$ 25†	276 $\pm$ 20†
Mean arterial blood pressure (mm Hg)	56 $\pm$ 2	55 $\pm$ 2	58 $\pm$ 2‡
Peripheral resistance (mm Hg $\cdot$ ml $^{-1} \cdot$ min $\cdot$ 100 gm)	2.07 $\pm$ 0.12	1.60 $\pm$ 0.12†	2.15 $\pm$ 0.14†
Uteroplacental vascular resistance (mm Hg $\cdot$ ml $^{-1} \cdot$ min $\cdot$ 100 gm of placenta)	0.22 $\pm$ 0.03	0.31 $\pm$ 0.05†	2.15 $\pm$ 0.03

\*There were no significant differences between the control and recovery values of any of these variables.

† $p < 0.05$  compared to the preceding value.

‡ $p < 0.01$  compared to the preceding value.

of these values had returned essentially to control levels 2 hours after the end of the ritodrine infusion (Table I).

Blood flows and the fractions of cardiac output distributed to the various organs (except placenta and myometrium) before, during, and 2 hours after the infusion of ritodrine are shown in Fig. 2, A and B. During ritodrine infusion, significant ( $p < 0.05$ ) increases occurred in the flows to carcass and myocardium and also in the proportion of cardiac output distributed to these organs. These two organs together accounted for 76% of the total increase in cardiac output. The gastrointestinal tract received a further 15% of the increment in cardiac output, although the fraction of cardiac output distributed to the gastrointestinal tract did not increase. The flows to most other organs

except the lungs, kidneys, and liver also increased but not significantly. The fraction of cardiac output distributed to the kidneys decreased ( $p < 0.01$ ) during ritodrine infusion, although absolute renal blood flow did not change. The proportions of microspheres reaching the lungs were 5.8% for the control injection and 4.3% for the ritodrine and recovery injections. Two hours after the end of the infusion the organ flows and cardiac output distribution were not significantly different from those at the end of the control period (Fig. 2, A and B). The pattern of change in the cardiac output and its distribution in the six animals with proper placement of the left ventricular catheter was similar to that found in the entire group.

Mean blood flow to the placentas decreased from  $28 \pm 6 \text{ ml} \cdot \text{min}^{-1}$  ( $272 \pm 27 \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{ gm}^{-1}$ ) at

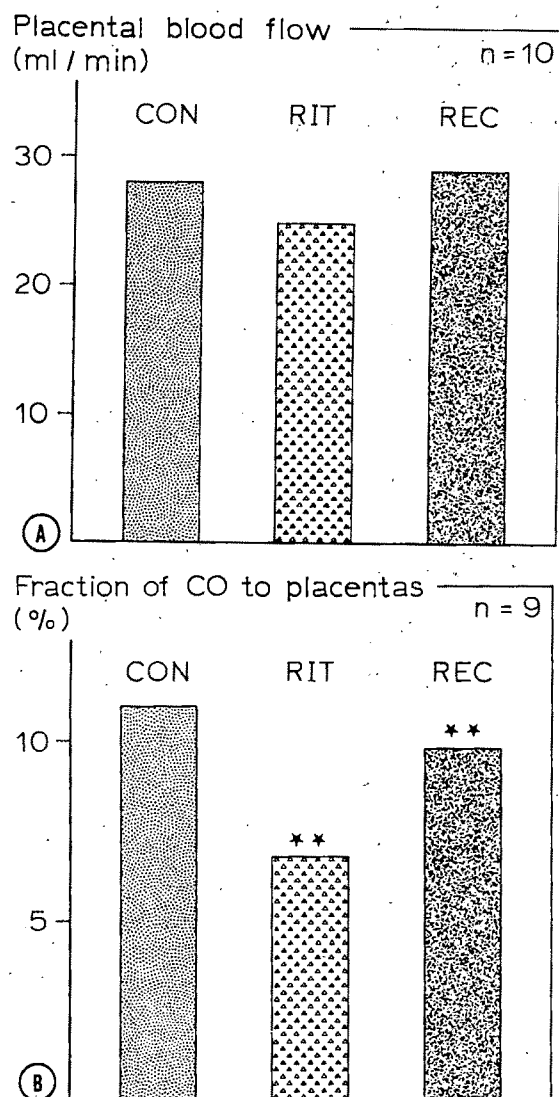


Fig. 3. Changes in maternal placental blood flow (A) and placental fraction of cardiac output (CO) (B) during and after infusion of ritodrine in pregnant guinea pigs. \*\* =  $p < 0.01$  compared to preceding value. CON, Control; RIT, ritodrine; REC, recovery.

the end of the control period to  $25 \pm 7 \text{ ml} \cdot \text{min}^{-1}$  ( $241 \pm 39 \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{ gm}^{-1}$ ) after 2 hours of ritodrine infusion (Fig. 3, A and B). The difference in absolute flows was not statistically significant, while the difference in flow per 100 gm was just significant at the 0.05 level. The placental fraction of cardiac output decreased from  $11\% \pm 3\%$  to  $7\% \pm 2\%$  ( $p < 0.01$ ). Uteroplacental vascular resistance increased ( $p < 0.05$ ) (Table I). All values had returned essentially to control levels 2 hours after the end of the infusion.

Maternal placental blood flow in the six animals with only living fetuses at the time of the experiments followed the same pattern shown by the entire group: average placental blood flow decreased from  $36 \pm 7$

before to  $33 \pm 8 \text{ ml} \cdot \text{min}^{-1}$  at the end of the ritodrine infusion and returned to  $37 \pm 9 \text{ ml} \cdot \text{min}^{-1}$  at the end of the recovery period. The corresponding values for flow per 100 gm were  $260 \pm 35$ ,  $242 \pm 54$ , and  $262 \pm 44 \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{ gm}$ . With the smaller number of animals, neither change reached statistical significance. The placental fraction of cardiac output decreased significantly in these six animals, however, from 15 to 9% ( $p < 0.05$ ). Uteroplacental vascular resistance in this group increased by 27% from  $0.26 \pm 0.05$  to  $0.33 \pm 0.09 \text{ mm Hg} \cdot \text{ml}^{-1} \cdot \text{min} \cdot 100 \text{ gm}$ , but this also did not reach statistical significance.

Blood flow to myometrium and cervix increased approximately 20%, from  $4.1 \pm 0.6$  to  $4.9 \pm 0.8 \text{ ml} \cdot \text{min}^{-1}$ . This change was not statistically significant. The myometrial fraction of cardiac output remained essentially constant,  $1.6\% \pm 0.02\%$  in the control period and  $1.4\% \pm 0.2\%$  during infusion of ritodrine.

Cardiac output and blood flows in the skin, carcass, and myometrium at the end of the control period were higher in the three animals with both live and dead fetuses than in any of the six animals carrying only live fetuses. These differences disappeared by the end of the ritodrine infusion.

As shown in Fig. 4, placental blood flows decreased consistently during ritodrine infusion in eight animals and increased consistently in one, and one animal showed both increases and decreases in individual placentas. The two animals with increases in placental flows were those that showed the greatest increase in cardiac output with infusion of ritodrine. We therefore investigated the possibility of a correlation between the changes in cardiac output and those in placental blood flow and vascular resistance. The proportional changes in placental blood flow were found to be positively ( $r = 0.85$ ,  $p < 0.01$ ), and those in placental vascular resistance negatively ( $r = -0.88$ ,  $p < 0.01$ ) correlated with those in cardiac output. Only the values for the placentas of live fetuses were used in this correlation.

Fig. 4 also shows that the blood flow to the placentas of four of the dead fetuses was unmeasurably low, while three placentas still demonstrated blood flows in the lower range of the values observed with live fetuses.

#### Comment

After the initial postoperative weight loss the average daily weight gain in our animals was similar to that observed by Sparks et al.<sup>12</sup> in their metabolic studies of pregnant guinea pigs. The range of maternal weights observed was appropriate for pregnant guinea pigs near term.<sup>13</sup>

Both the pretreatment cardiac output and the increase during ritodrine infusion were greater in the guinea pigs we studied under chronic conditions than the corresponding values found by Martensson et al.<sup>9</sup>

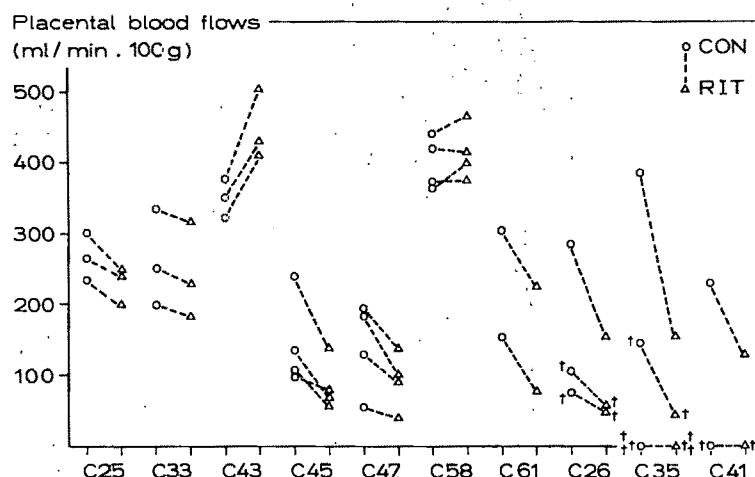


Fig. 4. Changes in blood flow to individual placentas during infusion of ritodrine to pregnant guinea pigs. † indicates placenta of dead fetus. C25, C33, etc., denote individual animals. CON, Control; RIT, ritodrine.

in their acute experiments. The pretreatment placental blood flows were also more than three times as high in the chronic as in the acute experiments, although the placental fractions of cardiac output did not differ greatly (11% in the present experiments compared to 8.8% and 11.2% in the two groups studied by Martensson et al.<sup>9</sup>). These differences probably reflect general cardiocirculatory depression from the pentobarbital anesthesia in the acute experiments. The lower dose of ritodrine used by Martensson et al.,  $12 \mu\text{g} \cdot \text{min}^{-1}$ , was approximately equal to that used by us. At this dosage level they observed a 22% increase in placental blood flow, whereas the placental fraction of cardiac output remained nearly constant. These findings were probably related to the low pretreatment placental flows, since in our chronically catheterized animals placental flow decreased during ritodrine infusion, both in milliliters per minute per 100 gm and as a fraction of the cardiac output. Although the initial level of myometrial blood flow was much higher in the chronic than in the acute experiments, the proportional increases in myometrial blood flow were similar: 20% in the present series and 25% in the observations of Martensson et al.<sup>9</sup> As noted, however, the change in myometrial blood flow in the present experiments was not statistically significant, and in absolute terms was much less than the decrease in placental blood flow, so that total uterine blood flow (myometrium plus placenta) decreased.

Our findings in the study of chronically catheterized pregnant guinea pigs thus agree in general with those reported earlier in studies with pregnant sheep (Ehrenkrantz et al.<sup>6</sup> and Brennan et al.<sup>7</sup>) that uterine blood flow decreases during infusion of ritodrine at rates of 8 and  $\sim 15 \mu\text{g} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ . With use of a lower dose of ritodrine ( $0.52$  to  $2.8 \mu\text{g} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ ), Siimes

et al.<sup>8</sup> found no change in placental or myometrial blood flows but a significant reduction in the placental and total uterine fractions of cardiac output. At this dosage level Siimes et al.<sup>8</sup> also noted a 31% increase in cardiac output and a 75% increase in blood flow to a sample of muscle, changes not greatly different from those found in our pregnant guinea pigs.

The pattern of change in cardiac output and its distribution observed during infusion of ritodrine also resembled generally that observed in similar experiments with isoproterenol in pregnant guinea pigs.<sup>14</sup> In both studies the cardiac output increased significantly, and the carcass, gastrointestinal tract, and myocardium received the greatest part of the increment. Total peripheral resistance decreased with both agents, and arterial blood pressure did not change significantly. The dose of isoproterenol used in the earlier study produced greater increases in maternal heart rate and cardiac output than were observed with ritodrine, 17% and 41% compared to 11% and 29%, respectively; this may account for many of the differences in results between the two groups of experiments.

The carcass received the greatest part of the increment in cardiac output with both ritodrine and isoproterenol. This was due not only to the large mass of the carcass relative to other organs, but also to the large increases in flow per unit weight, from 23 to 35  $\text{ml} \cdot \text{min}^{-1} \cdot 100 \text{ gm}^{-1}$  with ritodrine and from 19 to 33  $\text{ml} \cdot \text{min}^{-1} \cdot 100 \text{ gm}^{-1}$  with isoproterenol. According to current concepts,  $\beta$ -2 receptors are located extrajunctionally and presynaptically, whereas  $\beta$ -1 receptors are located postsynaptically in the sympathetic terminal junctions.<sup>15</sup>  $\beta$ -Adrenergic vasodilatation in skeletal muscle is considered to be the result of lactic acid production induced by  $\beta$ -2 receptors in the muscle cells. Thus a selective  $\beta$ -2 agonist such as ritodrine could be

expected to cause vasodilatation in the carcass (predominantly skeletal muscle) equal to that produced by the nonselective  $\beta$ -agonist isoproterenol, while causing relatively less stimulation of the rate and force of cardiac contraction. Both of these latter effects have been characterized as being more  $\beta$ -1 than  $\beta$ -2 in nature.<sup>15</sup>

Uteroplacental blood flow did not change after infusion of isoproterenol but decreased after ritodrine. Uteroplacental vascular resistance did not change with isoproterenol but increased with ritodrine. Since in the ritodrine experiments uteroplacental blood flow was positively correlated and resistance negatively correlated with the increment in cardiac output, the explanation for the difference in the effects of these two agents on uteroplacental blood flow and vascular resistance may lie in the greater average increase in cardiac output produced by isoproterenol. With ritodrine the large increase in carcass flow may have required reflex adjustments in flow distribution, including vasoconstriction in the uteroplacental vascular bed, in order to maintain blood pressure. The greater increase in cardiac output with isoproterenol may have made such redistribution of the cardiac output unnecessary.

Myometrial blood flow did not change significantly with ritodrine but increased 72% with isoproterenol. This difference is probably due to the  $\beta$ -1 activity of isoproterenol as opposed to the relative  $\beta$ -2 specificity of ritodrine.

Our results indicate that in the pregnant guinea pig  $\beta$ -adrenergic agents have no demonstrable vasodilator effect on the maternal vessels supplying the placenta. The correlations already cited between the magnitude of the cardiac output increase and the changes in uteroplacental blood flow and vascular resistance suggest that the effect of these agents on maternal placental blood flow depends on the balance between the increase in cardiac output and vasodilatation in the carcass.

We conclude that the major circulatory effects of ritodrine,  $15 \mu\text{g} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ , in the near term pregnant guinea pig are an increase in heart rate and cardiac output, and increases in blood flow to the carcass, heart, gastrointestinal tract, and some other organs. The effect on maternal placental blood flow depends on the balance between the degree of increase in cardiac output and the degree of vasodilatation in systemic vascular beds, especially the carcass.

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# Fetal responses to maternal infusions of angiotensin II

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It is unclear whether the fetus is affected by maternal infusions of angiotensin II; therefore we studied maternal and fetal responses ( $n = 9$ ) to angiotensin II (1.15, 2.29, 11.5  $\mu\text{g}/\text{min}$ ) infused 5 minutes into the vena cava of chronically instrumented sheep (129 to 137 days of gestation) while monitoring  $\text{PO}_2$ ,  $\text{PCO}_2$ , pH, heart rate, uterine blood flow, and arterial and umbilical venous pressures. Pregnant sheep demonstrated expected dose-related increases in mean arterial pressure and decreases in uterine blood flow ( $p < 0.05$ ). Increases in fetal mean arterial pressure also correlated with the maternal dose of angiotensin II ( $r = 0.77$ ,  $p < 0.001$ ). Fetal heart rate appeared to increase with 2.29  $\mu\text{g}/\text{min}$ ; however, bradycardia was observed with 11.5  $\mu\text{g}/\text{min}$  ( $p < 0.05$ ) and was associated with decreased  $\text{PaO}_2$ ,  $19.0 \pm 1.0$  to  $14.3 \pm 1.4$  mm Hg ( $p < 0.05$ ), increased  $\text{PaO}_2$  ( $p < 0.05$ ), and decreased umbilical venous  $\text{PO}_2$ ,  $31.4 \pm 2.3$  to  $27.0 \pm 1.9$  mm Hg. The decreases in  $\text{PO}_2$  correlated with decreases in uterine blood flow ( $r = 0.60$ ,  $p < 0.002$ , and  $r = 0.75$ ,  $p < 0.005$ , respectively). Nevertheless, changes in fetal mean arterial pressure also occurred in the absence of altered fetal oxygenation; thus decreased uterine blood flow and fetal oxygenation alone cannot explain the fetal cardiovascular responses. It is suggested that angiotensin II or an active metabolite may cross the ovine placenta. (AM J OBSTET GYNECOL 1986;154:195-203.)

**Key words:** Uterine blood flow, fetus, angiotensin II, fetal oxygenation

Alterations in vascular reactivity to several vasoconstrictor agents occur in pregnancies complicated by pregnancy-induced hypertension.<sup>1</sup> Gant et al.<sup>2</sup> have shown that the systemic pressor responses to infused angiotensin II may be significantly increased as early as 18 to 20 weeks of gestation in normotensive pregnant women destined to subsequently develop pregnancy-induced hypertension, whereas the relative refractoriness normally seen in pregnancy persisted in those who did not develop hypertension. It has been suggested from these observations that the pressor response to infused angiotensin II may provide a tool useful for screening primigravid women at risk of developing pregnancy-induced hypertension.<sup>3-5</sup>

Although information concerning the pressor response to infused angiotensin II in pregnant women is abundant, little is known about potential fetal effects of maternal infusions. This issue assumes importance, since angiotensin II, a potent vasoconstrictor, not only increases systemic vascular resistance but also increases

uterine vascular resistance and thus decreases uterine blood flow in a dose-dependent fashion.<sup>6,7</sup> This concern is further supported by observations in pregnant woman that decreases in uterine perfusion may occur during the systemic infusion of angiotensin II<sup>8</sup> and that fetal responses have been observed.<sup>9</sup> The possibility therefore exists that infused angiotensin II may affect placental perfusion, uterine oxygen delivery, and thus fetal well-being or that angiotensin II may cross the placenta; however, these concerns remain speculative.

In preliminary studies of the effects of infused angiotensin II on the cardiovascular system of pregnant sheep, we observed an apparent fetal response to maternal infusions of angiotensin II. In these experiments there appeared to be a dose-related rise in fetal mean arterial pressure and a decrease in fetal heart rate, responses typically associated with reductions in fetal oxygenation or infusions of vasoconstrictors.<sup>10,11</sup> Because of these observations, our knowledge of the uterine responses to infused angiotensin II, and conflicting reports of fetal effects during maternally administered angiotensin II, we sought to determine in near-term pregnant sheep whether fetal responses to maternal infusions of angiotensin II occurred and whether these responses correlate solely with alterations in uteroplacental blood flow.

## Material and methods

**Animal preparation.** Five chronically instrumented, pregnant ewes of mixed breed ( $51.4 \pm 7.2$  kg; range, 43 to 61 kg) bearing singleton fetuses ( $3.41 \pm 0.45$  kg;

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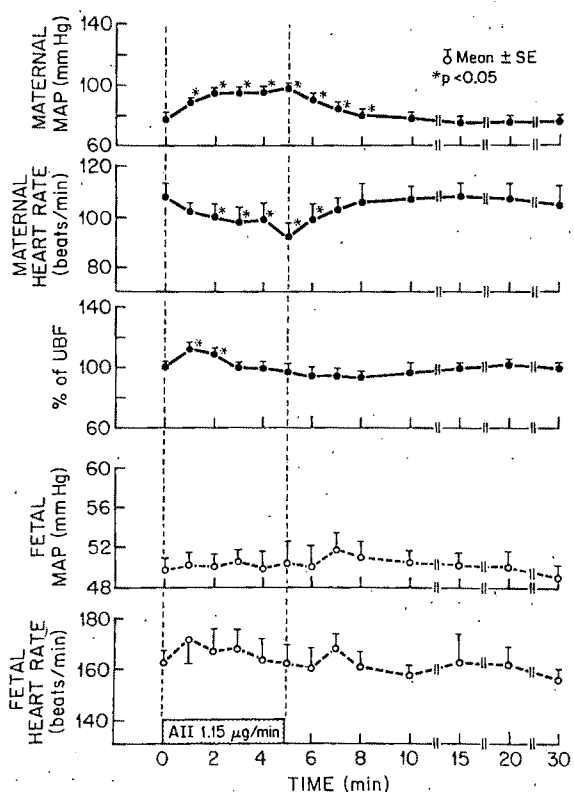


Fig. 1. Maternal and fetal cardiovascular responses to the maternal systemic infusion of 1.15  $\mu\text{g}/\text{min}$  of angiotensin II (AII); p values represent values compared to control by analysis of variance of repeated measures.

range, 2.91 to 4.13 kg) were used in this investigation. The studies were performed between 129 and 137 days of gestation ( $133 \pm 2.4$  days; term,  $144 \pm 3$  days). The surgical procedures performed on the ewe and the fetus have been described in detail.<sup>12</sup> To state them briefly, after induction of spinal anesthesia, the uterus was exposed through a midline abdominal incision, and one hind leg of the fetus was delivered through a small uterine incision. Polyvinyl catheters were inserted 6.5 cm into the fetal femoral artery, the tip lying in the lower aorta and representative of the umbilical artery, 12.0 cm into the femoral vein, the tip lying in the high inferior vena cava, and 3.0 cm into one umbilical vein near the insertion of the umbilicus into the abdominal wall, the tip lying within the common umbilical vein. Another catheter was positioned in the amniotic sac. The fetal leg was placed back into the amniotic sac; the uterus was then closed and returned to the abdominal cavity of the ewe. Ampicillin (250 mg) was instilled in the amniotic cavity through the amniotic catheter.

Electromagnetic flow probes (Micron Instruments Inc., Los Angeles, California) were implanted around both main uterine arteries. All fetal catheters and the flow probe leads were exteriorized through a stab wound in the abdominal fascia of the ewe and the ab-

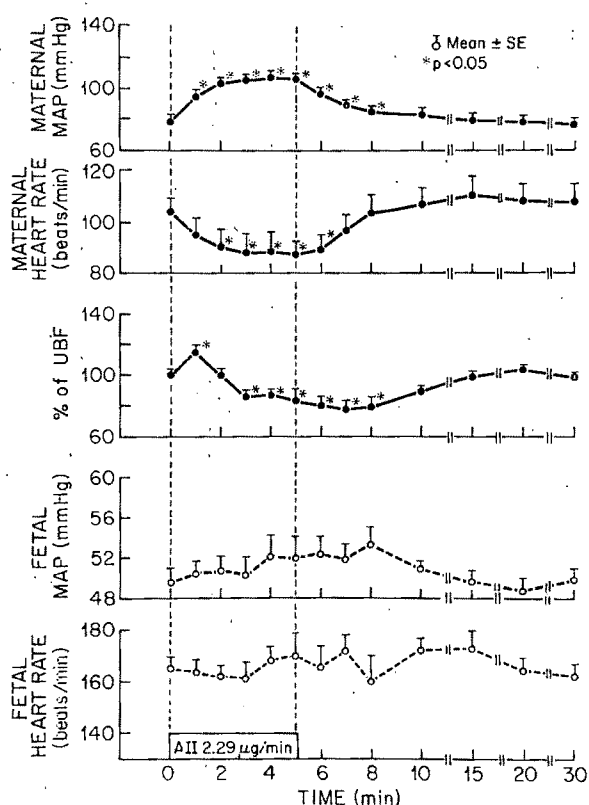


Fig. 2. Maternal and fetal cardiovascular responses to the maternal systemic infusion of 2.29  $\mu\text{g}/\text{min}$  of angiotensin II (AII). Statistical analysis is as noted in Fig. 1.

domen closed. Through separate groin incisions, catheters were inserted 15 cm into both maternal femoral arteries, the open ends lying just distal to the aortic bifurcation, and 30 cm into both maternal femoral veins, the tips lying in the inferior vena cava just below the diaphragm. The flow probe leads and all catheters were brought out to the flank through a subcutaneous tunnel and placed in a canvas pouch attached to the skin with steel pins. The catheters were flushed daily with 0.15 mol/L sodium chloride containing heparin (250 U/ml) and closed with sterile metal pins. The ewes were given intramuscular penicillin (500,000 U) and streptomycin (0.5 gm) on the day of surgery and each of the next 2 days. The fetus was given ampicillin (50 mg intravenously) every other day.

After the operation each ewe was maintained in a stall in the laboratory and given feed and water ad libitum. Throughout the study maternal weight remained constant or increased. The experiments were performed only after the animals were considered to have recovered from the trauma of the operation and anesthesia; no animal was studied before the seventh day after the surgical procedure. Four animals were studied twice, and experiments were done at least 48 hours apart; one animal was studied once.

**Experimental protocol.** Angiotensin II (Ciba-Geigy Corp., Summit, New Jersey) was diluted in sterile isotonic saline solution to a concentration of 3  $\mu\text{g}$  of base per milliliter. This solution was infused through one of the maternal venous catheters by a constant-infusion pump (Harvard Apparatus Co., Inc., South Natick, Massachusetts). In each experiment three doses of angiotensin II were studied (1.15, 2.29, and 11.5  $\mu\text{g}/\text{min}$ ) in a random sequence. Each infusion was maintained for 5 minutes, which results in a steady-state response,<sup>6,7</sup> while monitoring was performed for uterine blood flow, maternal and fetal mean arterial pressure, heart rate, amniotic fluid pressure, and mean umbilical venous pressure. After stopping the angiotensin II infusion, these parameters were monitored another 25 minutes, or until they had returned to preinfusion values. Blood samples for  $\text{PO}_2$ ,  $\text{PCO}_2$ , and pH were obtained from the maternal arterial and fetal arterial and umbilical venous catheters before the angiotensin II infusion and 5 minutes after starting the infusion. With the high dose of angiotensin II (11.5  $\mu\text{g}/\text{min}$ ), blood gases also were obtained at 5 and 15 minutes after the stopping of the angiotensin II infusion.

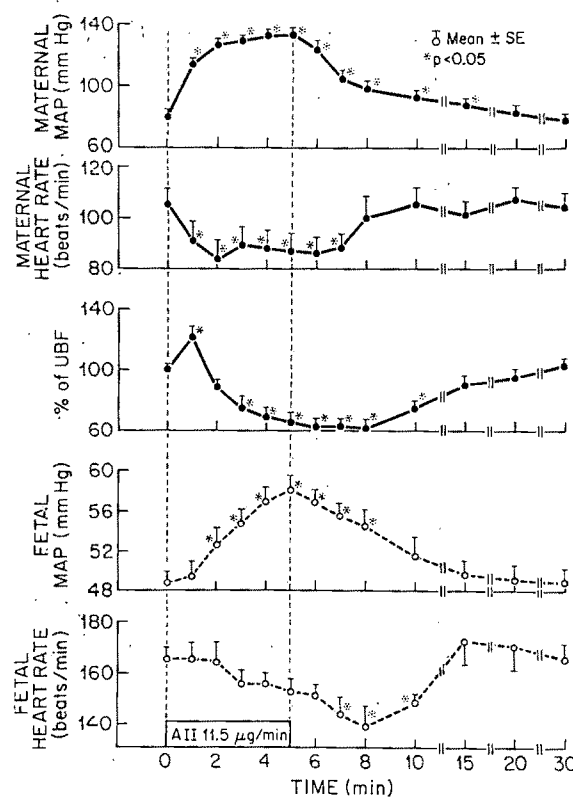
Pressures were monitored continuously through pressure transducers (Type 4-327-0 109, Bell & Howell, Pasadena, California) and the signals recorded on two-channel pen recorders (Brush, Model 220, Gould Inc., Cleveland, Ohio). Heart rates were obtained at intervals from a direct recording of the phasic signal from the tracings of arterial pressure. Uterine blood flow was continuously monitored with square-wave electromagnetic flowmeters (Model 1000, Micron Instruments Inc., Los Angeles, California) and recorded on a separate two-channel pen recorder. All recorders were electronically integrated.

Blood samples (0.5 ml) for blood gas and acid-base determination were obtained in 1.0 ml heparinized glass syringes and kept on ice until analyzed with use of a blood gas analyzer (Model 113, Instrumentation Laboratory, Lexington, Massachusetts). The hematocrit was measured on these samples by the micro-method of Wintrobe.

**Analysis of data.** Statistical analysis was performed with use of analysis of variance of repeated measures followed by the Student-Newman-Keuls multiple range test. Reported differences are considered significant at  $p < 0.05$ . All data are reported as the mean and one standard error.

## Results

The maternal responses in mean arterial pressure, heart rate, and uterine blood flow are compared with those of fetal mean arterial pressure and heart rate before, during, and after stopping the 5-minute maternal infusion of angiotensin II (1.15, 2.29, and 11.5



**Fig. 3.** Maternal and fetal cardiovascular responses to the maternal systemic infusion of 11.5  $\mu\text{g}/\text{min}$  of angiotensin II (AII). Statistical analysis is as noted in Fig. 1.

$\mu\text{g}/\text{min}$ ) in Figs. 1 through 3. As might be predicted from previous studies,<sup>6,7</sup> there was a dose-dependent increase in maternal mean arterial pressure. This rise in pressure was significant within 1 minute after starting the infusion of each dose of angiotensin II, and a steady state was achieved with increases with the three doses of angiotensin II of 25%, 36%, and 67%, respectively, above control values ( $p < 0.05$ ). After stopping the angiotensin II infusion, mean arterial pressure gradually returned to baseline values with each dose of angiotensin II; however, the time interval also was dose dependent, for example, with a dose of 11.5  $\mu\text{g}/\text{min}$  (Fig. 3) the mean arterial pressure remained significantly greater than control values for up to 15 minutes after stopping of the infusion and was not different from control values until 20 minutes.

Associated with the rise in maternal mean arterial pressure was a fall in maternal heart rate at all three doses of angiotensin II. The fall in heart rate was significant by 2 minutes with each dose, and a steady state was evident thereafter, persisting for 1 to 2 minutes after stopping the angiotensin II infusion. Heart rates gradually returned to values not different from control values despite persistent elevations in mean arterial pressure at all three doses of angiotensin II.

Because of the variability in baseline values for uter-

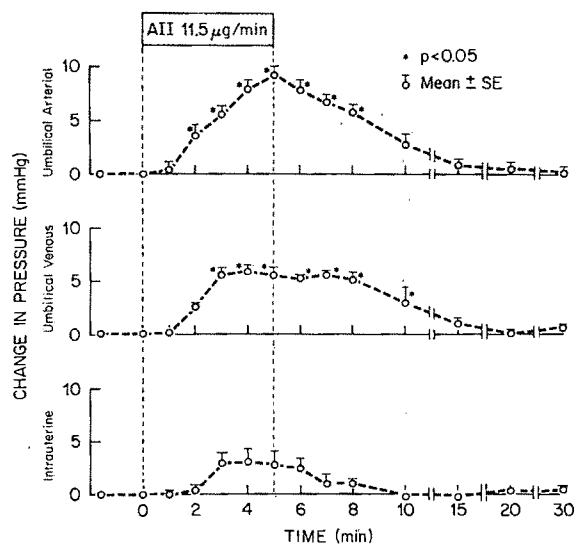


Fig. 4. Relationship between changes in mean umbilical arterial, umbilical venous, and intrauterine pressures during the maternal systemic infusion of 11.5  $\mu\text{g}/\text{min}$  of angiotensin II (AII). Statistical analysis is as noted in Fig. 1.

ine blood flow between animals, the uterine responses to infused angiotensin II are presented as the percent change from control values. It is obvious from the small variability at each time point, that all animals responded similarly. As previously reported,<sup>6,7</sup> there was a typical time-related biphasic response in uterine blood flow with each dose of angiotensin II, that is, uterine blood flow rose significantly at 1 minute into the angiotensin II infusion ( $p < 0.05$ ) and subsequently fell, reaching a steady state by 3 minutes. Both the increases and decreases (measured in the steady state) are dose dependent: There was no fall with the dose of 1.15  $\mu\text{g}/\text{min}$ , whereas uterine blood flow fell  $\sim 20\%$  with the 2.29  $\mu\text{g}/\text{min}$  dose and 35% to 40% with the 11.5  $\mu\text{g}/\text{min}$  dose. Although uterine blood flow returned to control values, the decreases persisted 5 minutes after stopping the infusion with the 2.29  $\mu\text{g}/\text{min}$  dose and at least 10 minutes with the 11.5  $\mu\text{g}/\text{min}$  dose, resulting in similar durations of change in uterine blood flow and maternal mean arterial pressure.

In the fetal compartment there were no statistically significant changes in mean arterial pressure with either 1.15 or 2.29  $\mu\text{g}$  of angiotensin II per minute (Figs. 1 and 2, respectively); there was, however, a tendency for fetal mean arterial pressure to rise at 4 to 5 minutes after initiation of the infusion of 2.29  $\mu\text{g}/\text{min}$  and for this to persist until 3 minutes after its cessation. With infusion of 11.5  $\mu\text{g}$  of angiotensin II per minute (Fig. 3), fetal mean arterial pressure was significantly elevated 2 minutes into the maternal angiotensin II infusion from a control value of  $49 \pm 1.1$  mm Hg. Although mean arterial pressure appears to continue to rise until the end of the angiotensin II infusion, when

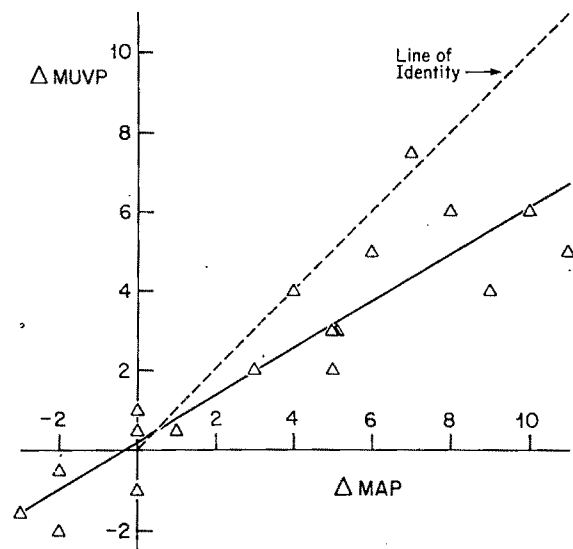


Fig. 5. Relationship between the change in fetal mean arterial pressure ( $\Delta\text{MAP}$ ) and mean umbilical venous pressure ( $\Delta\text{MUVP}$ ) at 5 minutes during the maternal systemic infusion of three doses of angiotensin II (1.15, 2.29, and 11.5  $\mu\text{g}/\text{min}$ ). The regression equation is noted in the text.

the mean increase was  $9.2 \pm 0.98$  mm Hg or 20%, there were no differences between values from 3 to 8 minutes ( $p > 0.05$ , analysis of variance). Fetal mean arterial pressure returned to values not different from control values 5 minutes after stopping the angiotensin II infusion. In light of the apparent dose-related increase in fetal mean arterial pressure, we examined the relationship between the change in mean arterial pressure at 5 minutes ( $y$ ) and the log of the rate of infusion of angiotensin II per kilogram of maternal weight ( $x$ ). The equation describing this relationship,  $y = -11.2 + 8.54(\log x)$ ,  $r = 0.77$ ,  $n = 27$ , was significant,  $p < 0.001$ .

Although there were no significant alterations in fetal heart rate with the two lowest doses of angiotensin II, mean heart rate rose from 165 to  $\sim 170$  bpm between 5 and 8 minutes with a dose of 2.29  $\mu\text{g}/\text{min}$ . Fetal bradycardia was evident within 3 minutes of starting 11.5  $\mu\text{g}$  of angiotensin II per minute, but significance ( $p < 0.05$ ) was not obtained until 2 minutes after stopping the infusion, having decreased 13%, from  $165 \pm 5$  to  $144 \pm 6$  bpm. Fetal heart rate returned to control levels at 15 minutes, simultaneously with the return of fetal mean arterial pressure.

We were able to obtain continuous pressure recordings from the common umbilical venous catheter during 18 infusions of angiotensin II; the mean control value was  $11 \pm 0.63$  mm Hg. No significant changes in mean umbilical venous pressure were observed with the two lower doses of angiotensin II. However, by 3 minutes into the angiotensin II infusion of 11.5  $\mu\text{g}/\text{min}$



**Table I.** Effect of maternal infusions of angiotensin II (11.5 µg/min) on fetal umbilical arterial (n = 9) and venous (n = 4) blood gases and pH

	Control	5 min	10 min	20 min
Umbilical arterial				
PO <sub>2</sub> (mm Hg)	18.98 ± 0.98	16.39 ± 1.23*	14.28 ± 1.43*	18.85 ± 1.17
PCO <sub>2</sub> (mm Hg)	44.43 ± 1.27	47.21 ± 1.63	49.34 ± 2.26*	46.53 ± 1.54
pH	7.39 ± 0.008	7.37 ± 0.011	7.36 ± 0.007*	7.38 ± 0.012
Umbilical venous				
PO <sub>2</sub> (mm Hg)	31.40 ± 2.25	25.00 ± 1.04	26.92 ± 1.92	30.63 ± 3.5
PCO <sub>2</sub> (mm Hg)	37.82 ± 2.5	40.00 ± 1.3	39.57 ± 1.23	38.00 ± 1.5
pH	7.43 ± 0.03	7.43 ± 0.01	7.41 ± 0.02	7.42 ± 0.01

Values are the mean ± SE.

\*p &lt; 0.05.

(Fig. 4) mean umbilical venous pressure increased  $5.42 \pm 0.86$  mm Hg ( $p < 0.05$ ) from control levels and remained significantly elevated until 5 minutes after stopping the infusion. This pattern of response parallels that observed with fetal mean arterial pressure except that significant increases are delayed 1 minute when compared to mean arterial pressure. Because of the similarity in the patterns of response in fetal mean arterial pressure (MAP) and mean umbilical venous pressure (MUV), we examined the relationship between the changes in these two variables at all time points at which simultaneous measurements were available ( $n = 78$ ). The relationship was linear, and the correlation coefficients were 0.66, 0.61, and 0.81 for 1.15, 2.29, and 11.5 µg of angiotensin II per minute, respectively ( $p < 0.001$ ). For each dose the slope was statistically different from zero; furthermore, the higher the dose, the greater the slope and the greater the tendency toward a line of identity. Because the fetal responses were maximum (or in a steady state) at 5 minutes into each infusion of angiotensin II, we also examined this relationship at this time (Fig. 5). Six paired measurements for each dose of angiotensin II were available ( $n = 18$ ). The regression equation for the relationship was  $\Delta\text{MUV} = 0.28 \pm 0.58(\Delta\text{MAP})$ , where  $r = 0.91$ ,  $p < 0.001$ . The regression line was not different from the line of identity.

We also monitored mean amniotic fluid pressure (Fig. 4) during these studies; before the infusion of angiotensin II the mean value was  $5.42 \pm 0.47$  mm Hg. Although amniotic fluid pressure rose slightly with each dose of angiotensin II studied, these changes were not statistically significant ( $p > 0.05$ ).

Maternal and fetal arterial blood gases and pH were unchanged during the maternal infusion of 1.15 and 2.29 µg of angiotensin II per minute. With 11.5 µg/min of angiotensin II, maternal PaO<sub>2</sub> fell slightly from  $110 \pm 2.0$  mm Hg to  $95.3 \pm 3.3$  mm Hg ( $p < 0.05$ ), a value well within the physiologic range for pregnant sheep; there were no changes in pH<sub>a</sub> or PaCO<sub>2</sub>. The changes in fetal blood gases and pH with the high dose

of angiotensin II are presented in Table I. Fetal umbilical arterial PO<sub>2</sub> decreased significantly from control values at the end of the maternal angiotensin II infusion (5 minutes) and decreased further at 5 minutes after stopping the infusion,  $p < 0.05$ . At 15 minutes after stopping the infusion, PaO<sub>2</sub> had returned to values not different from control. Fetal PaCO<sub>2</sub> rose  $10 \pm 2.4\%$  above control values ( $p < 0.05$ ), when the fall in PaO<sub>2</sub> was greatest, and returned to control levels 15 minutes after stopping the angiotensin II infusion. The pH fell and rose in a pattern probably reflecting the change in PaCO<sub>2</sub>. In four experiments we were able to obtain blood samples from the umbilical venous catheter (Table I). The trend was similar to that observed in the arterial samples; however, because of the small sample size, these changes were not significant.

To determine whether these changes in fetal oxygenation may have been related to the angiotensin II-induced alterations in uterine blood flow, we examined the relationship between the percent change in flow and the change in both fetal arterial and umbilical venous PO<sub>2</sub> (Fig. 6). In both cases the relationship was significant, oxygen tension decreasing as uterine blood flow (UBF) fell. For fetal arterial PO<sub>2</sub> the linear regression equation was  $\Delta\text{PaO}_2 = -0.098 + 0.062(\%\Delta\text{UBF})$ , where  $r = 0.60$ ,  $p < 0.002$ ; for umbilical venous PO<sub>2</sub> the regression equation was  $\Delta\text{PaO}_2 = 1.98 + 0.201(\%\Delta\text{UBF})$ , where  $r = 0.75$ ,  $p < 0.005$ . These relationships were not different from each other ( $p > 0.05$ ).

### Comment

The maternal cardiovascular responses to systemic infusions of angiotensin II have been studied in some detail. It is widely accepted that although refractoriness to the vasoconstrictor effects of angiotensin II normally develops during pregnancy,<sup>2,6</sup> it remains a potent vasoconstrictor in both the systemic and, importantly, the uterine vascular beds.<sup>6,7</sup> However, whether or not simultaneous fetal cardiovascular responses occur during maternal angiotensin II infusions remains controversial. For example, although some have reported

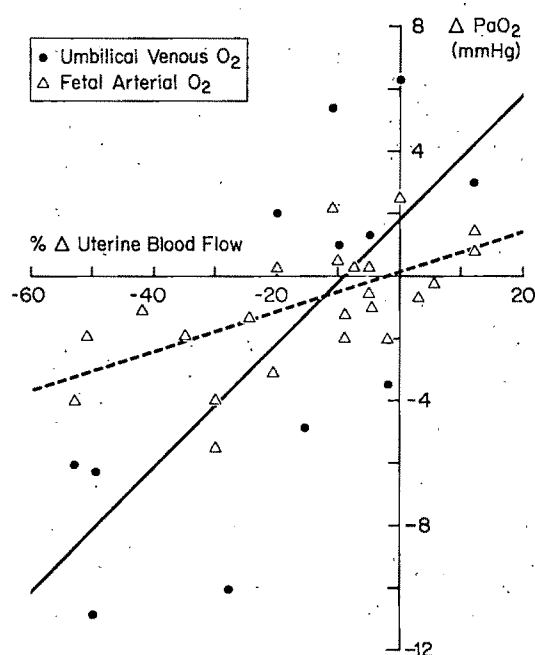


Fig. 6. Relationship between the percent change in uterine blood flow and the change in umbilical venous (●—●) and fetal arterial (Δ---Δ) oxygen tension during maternal systemic infusions of angiotensin II. See text for details.

increased frequency of fetal movements, fetal tachycardia, and increased uterine tone during systemic infusions of angiotensin II in women studied at term or after term,<sup>9</sup> others observed no such changes during infusions (0.4 to 1.43  $\mu\text{g}/\text{min}$ ) in normotensive gravid women between 27 and 37 weeks of gestation.<sup>5</sup> Similar discrepancies have been reported in animal experiments. Behrman and Kittinger,<sup>13</sup> studying the pregnant rhesus monkey during anesthesia and surgical stress, observed no fetal cardiovascular changes during constant maternal angiotensin II infusions ranging from 1 to 10  $\mu\text{g}/\text{min}$ . Similarly, no fetal effects were observed in acute studies of bolus doses of angiotensin II in pregnant sheep.<sup>14, 15</sup> In contrast, however, Bruce et al.<sup>16</sup> reported an apparent dose-dependent rise in fetal mean arterial pressure and fall in heart rate during the constant infusion of angiotensin II into pregnant sheep studied at a time remote from anesthesia and surgical stresses, factors known to influence hemodynamic responses to both vasodilators and vasoconstrictors. Because of these conflicting results and our preliminary observations of fetal cardiovascular responses during maternal infusions of angiotensin II, we performed the present studies.

In the present experiments we examined three doses of infused angiotensin II, two of which would result in physiologic plasma concentrations (1.15 and 2.29  $\mu\text{g}/\text{min}$ ) and one that would result in a pharmacologic concentration (11.5  $\mu\text{g}/\text{min}$ ). They also were chosen

because they covered a broad spectrum of the uterine and systemic dose-response curves previously reported<sup>6, 7</sup> and thus would allow examination of the relationship between fetal responses and alterations in maternal uterine blood flow. As previously reported,<sup>6, 7</sup> there was a dose response for maternal mean arterial pressure, heart rate, and uterine blood flow. The rise in uterine blood flow seen during the first minute of the infusion of each dose of angiotensin II is consistent with the observation that the uteroplacental vascular bed responds less rapidly to the vasoconstrictor effects of angiotensin II than the systemic vasculature overall, resulting in a rise in perfusion pressure (mean arterial pressure) that exceeds that of uterine vascular resistance and summates as an increase in uterine blood flow at this time point.<sup>7</sup> The dose-dependent reduction in uterine blood flow during the steady-state response is similarly related to the relationship between changes in perfusion pressure and uterine vascular resistance<sup>7</sup> and is not a reflection of a decrease in cardiac output as suggested by others.<sup>16</sup> The duration of the reduction in uterine blood flow also was dose related, remaining significantly decreased for up to 10 minutes after stopping the infusion of 11.5  $\mu\text{g}/\text{min}$  as compared to 5 minutes with 2.29  $\mu\text{g}/\text{min}$ . Although dose-response curves could not be generated, Gant et al.<sup>8</sup> have observed a fall in the placental clearance of dehydroisoandrosterone sulfate, reflective of placental blood flow, during the systemic infusion of angiotensin II in gravid women. Thus the potential for a decrease in uterine blood flow also exists in women infused with angiotensin II.

Associated with the maternal cardiovascular responses to infused angiotensin II was the simultaneous development of a dose-related rise in fetal mean arterial pressure and variable changes in fetal heart rate (Figs. 1 through 3). It might be argued that a significant change in fetal mean arterial pressure occurred only during the infusion of a pharmacologic dose of angiotensin II; however, when the relationship between the dose of angiotensin II and fetal mean arterial pressure was examined at 5 minutes of the infusion (the steady state) a highly significant correlation ( $r = 0.77$ ,  $p < 0.001$ ) was found. Bruce et al.<sup>16</sup> observed a similar, although weaker correlation and, as in the present report, reported that fetal mean arterial pressure was unchanged at doses of  $<1.0$   $\mu\text{g}/\text{min}$ . The response of fetal heart rate, on the other hand, did not correlate with either fetal mean arterial pressure or the dose of infused angiotensin II but did fall with 11.5  $\mu\text{g}$  of angiotensin II per minute. Bruce et al.<sup>16</sup> also did not find a significant change in fetal heart rate until the dose of angiotensin II exceeded 5  $\mu\text{g}/\text{min}$ .

Although it appears that there are dose-related fetal cardiovascular changes during maternal infusions of

angiotensin II in animals studied at a time remote from anesthesia and surgery, an explanation is not clearly apparent. Factors that may account for these observations include (1) placental transfer of angiotensin II or its active metabolites, (2) changes in uterine oxygen delivery and thus fetal oxygen uptake, and (3) the relationship between alterations in maternal placental and fetal umbilical perfusion. The question of placental transfer of angiotensin II remains cloudy. In earlier studies,<sup>3-15</sup> it was concluded that maternal to fetal transfer of angiotensin II did not occur because of an absence of fetal responses. However, as noted earlier, these observations may have reflected the use of stressed animal preparations; our observations and those of Bruce et al.<sup>16</sup> negate these conclusions. Evidence for placental transport of angiotensin II may be obtained from several studies. For example, Pipkin and O'Brien<sup>17</sup> reported that administration of saralasin, a competitive angiotensin II-receptor antagonist, to pregnant sheep resulted in a sixfold rise in maternal plasma levels of angiotensin II from 68 to 400 pg/ml (a value that might be expected with our lower doses of angiotensin II<sup>18</sup>) and an increase in fetal diastolic blood pressure. They also noted that fetal plasma angiotensin II concentrations rose in four of seven animals studied. It is unclear, however, whether the increases in fetal blood pressure occurred in the same animals that exhibited elevations in plasma angiotensin II levels. These investigators also have reported the recovery of radioactivity in the uterine venous effluent of guinea pigs after injecting <sup>125</sup>I-labeled angiotensin II into the umbilical artery.<sup>19</sup> However, they suggested that the recovered label was associated with a fragment that did not include the C-terminal phenylalanine residue and thus could not result in vasoconstrictor activity. It is unclear if the same metabolite would occur if transfer were from mother to fetus. In similar studies performed in pregnant sheep, infusions of angiotensin II for 5 to 7 minutes into the fetal umbilical artery resulted in a rise in maternal arterial concentrations of angiotensin II and a fall in plasma renin activity.<sup>20</sup> Since most radioimmunoassays for angiotensin II cross-react with its metabolites, for example, angiotensin III, it is unclear what fragments were measured. Nonetheless, evidence exists that angiotensin II may cross the placenta of several species in either direction; whether or not it is in an active form or is responsible for the fetal responses reported is unclear. Moreover, whether this pertains to the human also must be clarified, especially in light of recent evidence of angiotensinases in the microvilli of human placentas.<sup>21</sup>

If maternal-placental transfer of infused angiotensin II occurs, the observed fetal responses should be similar to those reported to occur during fetal infusions. In the present study and that of Bruce et al.<sup>16</sup> a dose-

related rise in fetal mean arterial pressure occurred, a finding consistent with observations of dose-dependent pressor responses during either bolus or constant infusions of angiotensin II into fetal sheep.<sup>11, 20, 22, 23</sup> We did not observe any significant correlations with or change in fetal heart rate except with the highest dose of angiotensin II, with which fetal bradycardia occurred, findings also similar to those of Bruce et al.<sup>16</sup> In contrast to the pressor responses, however, the reported fetal heart rate responses to infused angiotensin II are somewhat confusing; for example, reflex bradycardia,<sup>11</sup> transient bradycardia followed by tachycardia,<sup>20</sup> and tachycardia<sup>22</sup> have all been reported. More recently, Iwamoto and Rudolph<sup>23</sup> have shown in normoxic fetal sheep that despite a persistent increase in mean arterial pressure there is a transient fall in heart rate within the first 5 minutes of a constant angiotensin II infusion and that tachycardia develops thereafter. They suggested that the decrease was reflex in nature and due to the initial abrupt rise in mean arterial pressure, as suggested by Berman et al.,<sup>11</sup> whereas the subsequent tachycardia was a consequence of the predominant chronotropic effects of angiotensin II. It therefore is possible that the bradycardia observed by us at 3 to 4 minutes with a dose of 11.5 µg of angiotensin II per minute (Fig. 3) reflects this reflex bradycardia whereas the tendency for heart rate to rise with a dose of 2.29 µg/min in the presence of a relatively small increase in mean arterial pressure (Fig. 2) is reflective of the chronotropic effects of angiotensin II. Such a variable response would account for the lack of a correlation between fetal heart rate and maternal angiotensin II noted by us and others.

Acceptance of the above conclusions requires that another mechanism be postulated for the continued fall in heart rate seen with a dose of 11.5 µg/min. This may relate to the progressive decrease in fetal arterial and umbilical venous PO<sub>2</sub> seen after completing the angiotensin II infusion. The association of relative fetal hypoxemia with the continued fall in fetal heart rate suggests the release of catecholamines which would result in bradycardia,<sup>10, 11, 22</sup> while the initial fall in heart rate is reflexive. It does not rule out, however, the placental transfer of angiotensin II.

In view of the above discussion, the role of uterine blood flow, uterine oxygen delivery, and thus fetal oxygenation must be addressed as they pertain to the observed fetal cardiovascular changes. It has been suggested that an enormous "reserve" in uterine blood flow and fetal oxygen delivery is available to the fetus and that decreases in uterine blood flow of >50% are necessary before significant changes in fetal oxygen uptake occur.<sup>24</sup> In the present studies the mean decrease in uterine blood flow with the highest dose of angiotensin II was 40%, and individual values rarely exceeded 50%.

Moreover, the placental vascular bed of the sheep is extremely refractory to the vasoconstrictor effects of angiotensin II, and placental blood flow is decreased only 15% with a dose of 11.5  $\mu\text{g}/\text{min}$ .<sup>25</sup> This differential response may not occur with decreases in uterine blood flow induced by a vascular occluder, as was used to assess the relationship between uterine blood flow and fetal oxygenation, and would result in proportional falls in placental perfusion and total uterine blood flow.<sup>24</sup> Nonetheless, we did find a significant correlation between the fall in uterine blood flow and that of fetal umbilical venous and arterial  $\text{PO}_2$  (Fig. 6); however, we do not know whether fetal oxygen uptake was altered. Thus it is unclear whether the observed fall in fetal oxygen is the result of only a decrease in maternal uterine blood flow.

Angiotensin II is a potent vasoconstrictor of the fetal umbilical vascular bed,<sup>11, 23</sup> and it has been suggested that since fetal placental blood flow accounts for ~40% of cardiac output, any increase in umbilical vascular resistance would result in a rise in fetal mean arterial pressure.<sup>11</sup> Therefore, if placental transfer of angiotensin II or an active metabolite such as angiotensin III occurred, there would be a rise in umbilical vascular resistance and an increase in mean arterial pressure. This also would cause mean umbilical venous pressure to rise. At the higher dose of angiotensin II the occurrence of both a 40% fall in uterine blood flow and a significant decrease in umbilical blood flow could result in the observed decrease in fetal oxygenation and, in particular, the fall in umbilical venous  $\text{PO}_2$ . Moreover, it might even be suggested that a mismatch in fetal-placental and maternal-placental blood flows occurs, adding to the observed fall in fetal  $\text{PO}_2$ . This hypothesis is consistent with observations recently reported by Wilkening and Meschia.<sup>26</sup>

We have demonstrated that fetal responses to maternal infusions of angiotensin II occur with physiologic and pharmacologic doses of the hormone. We also have provided evidence that there indeed may be placental transfer of angiotensin II, but this evidence is indirect. Additionally, we conclude that these effects on the fetal cardiovascular system may in part be mediated through vasoconstriction of the fetal placental vasculature, which in association with a fall in uterine blood flow has the potential to result in decreased fetal oxygenation. Whether or not this pertains to the human remains to be seen.

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## The effect of antenatal dexamethasone on maternal and fetal retinol-binding protein

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Sixteen rhesus monkeys received 0.1 to 15 mg/kg of antenatal dexamethasone at 132 days' gestation; seven control animals received placebo. At 135 days' gestation they underwent cesarean section, and maternal and fetal serum was assayed for retinol-binding protein. Fetal and maternal concentrations of retinol-binding protein increased after dexamethasone ( $p < 0.05$ ) and there was a trend for fetal levels of retinol-binding protein to increase with increasing dosage ( $p < 0.01$ ). Whether the elevation of retinol-binding protein in response to antenatal dexamethasone is a desirable side effect is not clear at this time. (*AM J OBSTET GYNECOL* 1986;154:203-5.)

**Key words:** Retinol-binding protein, antenatal dexamethasone, vitamin A

The vitamin A (retinol) transport system in several primate species is similar to that in humans.<sup>1-3</sup> A specific retinol-binding protein is synthesized in the liver and mobilizes retinol from the liver, its principal storage site. After hepatic secretion, the retinol-binding protein-retinol complex circulates in blood further complexed with plasma transthyretin (commonly called prealbumin). When retinol is delivered to target tissues, the free retinol-binding protein is then rapidly catabolized and excreted by the kidney.<sup>4</sup> The turnover of retinol-binding protein in vivo is rapid, with a biologic half-life of 11.5 hours in adult humans<sup>4</sup> and 6.6 hours in cynomolgus monkeys.<sup>2</sup>

Adequate retinol nutritional status allows normal secretion of retinol-binding protein from liver, while retinol deficiency causes retinol-binding protein to accumulate in the liver and plasma levels to fall.<sup>5</sup> Early reports, before isolation methods for retinol-binding protein were available, suggested that adrenocortical

hormones could accelerate retinol mobilization from the liver.<sup>6-8</sup> More recently, dexamethasone was shown to stimulate the release of retinol-binding protein from cultured rat liver cells.<sup>9</sup>

Although the function of vitamin A (retinol) in the developing fetus is largely unexplored, the possible role of retinol in bronchopulmonary dysplasia after neonatal respiratory distress syndrome has been suggested.<sup>10</sup> Antenatal maternal steroids are used clinically to promote fetal lung maturation.<sup>11</sup> Since steroids potentially have a wide variety of effects on maternal and fetal systems besides lung maturation, these other effects should be assessed so their benefit/risk ratio can be determined.<sup>11</sup> The objective of our study was to test the hypothesis that antenatal maternal steroid (dexamethasone) given to accelerate fetal lung maturity stimulates the secretion of retinol-binding protein, resulting in increased levels of serum retinol-binding protein in both mother and fetus.

### Material and methods

Sixteen pregnant rhesus monkeys (*Macaca mulatta*) were given an intramuscular bolus of dexamethasone sodium phosphate (Organon Inc., West Orange, New Jersey) ranging in amount from 0.1 to 15 mg/kg at 132 days' gestation ( $\pm 1$  day). Seven control animals received saline solution instead of dexamethasone at 132

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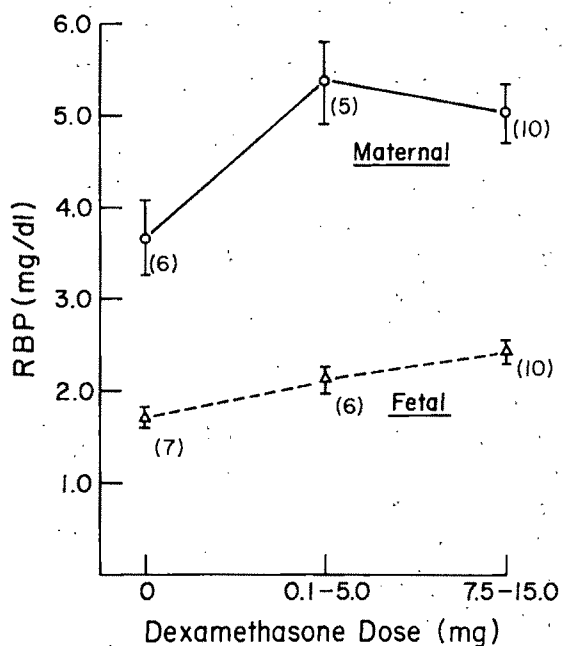


Fig. 1 Maternal (O) and fetal ( $\Delta$ ) concentration of retinol-binding protein at 135 days' gestation. The bars represent  $\pm 1$  SE of the mean. Samples are grouped by antenatal dexamethasone dose. Serum was not available for one control and one dexamethasone mother.

days' gestation. At 135 days' gestation, the mothers were given intramuscular ketamine hydrochloride (10 to 15 mg/kg; Parke-Davis, Detroit, Michigan) sedation and nitrous oxide-oxygen (4 L/min:2 L/min) for inhalation anesthesia. A cesarean section was then performed as previously described<sup>12</sup> and samples of fetal umbilical arterial and maternal blood were obtained for serum retinol-binding protein determination. Thirteen additional control animals (saline solution injection) underwent the same protocol except they received saline solution injection 3 days before cesarean delivery at the following gestational ages: one at 125 days, three at 145 days, six at 155 days, and three at 165 days. The animals were cared for at the Wisconsin Regional Primate Research Center in accordance with National Institutes of Health and United States Department of Agriculture guidelines.

Serum was separated and samples were frozen at  $-70^{\circ}\text{C}$  until use. Samples were analyzed within 14 months of their being drawn. Retinol-binding protein was measured with the use of LC-Partigen (Calbiochem-Behring, La Jolla, California) quantitative radial immunodiffusion based on published methods.<sup>13</sup> Previous work has shown that retinol-binding protein from the rhesus monkey cross reacts with human retinol-binding protein antibody.<sup>1</sup> An analysis of variance on duplicate samples with this method showed the intra-subject variability to be  $<5\%$  in our hands. Thirteen samples underwent repeat analysis after being frozen

for 11 to 29 months and failed to show a consistent or significant change in retinol-binding protein concentration (unweighted average slope of 0.014 per month,  $p > 0.10$ ).

**Statistical methods.** Correlations were done with Pearson's correlation coefficient and are expressed as an  $r$  value. Groups were created, based on the dexamethasone dose received, and group retinol-binding protein values were compared by means of an analysis of variance. Trends were also evaluated by regression analysis according to the absolute dexamethasone dose received (data not grouped). Further regression analysis was done for trends with the use of an adjustment for the age of the sample at time of analysis.

### Results

The average concentration of retinol-binding protein in maternal serum from control monkeys at 135 days' gestation was approximately twice that of fetal serum ( $3.66 \pm 0.41$  versus  $1.71 \pm 0.11$  mg/dl, mean  $\pm$  SEM). The correlation between maternal and fetal pairs for retinol-binding protein was weak for the control animals across all gestational ages ( $r = 0.17$ ). There was only a modest correlation between fetal and maternal values from animals that received dexamethasone ( $r = 0.29$ ). There was no significant trend in fetal or maternal retinol-binding protein concentrations with increasing gestational age.

The concentration of retinol-binding protein increased significantly ( $p < 0.05$ ) in both maternal and fetal serum in response to maternal dexamethasone administration (Fig. 1). Trend analysis showed that fetal retinol-binding protein increased with increasing doses of dexamethasone, and the significance ( $p < 0.01$ ) was not affected by adjustment for age of the sample at analysis. Although maternal retinol-binding protein levels increased after antenatal dexamethasone, there was no consistent trend for increased maternal retinol-binding protein with increasing dexamethasone doses.

### Comment

Vitamin A is potentially very important to the fetal and neonatal lung. It has an essential role in maintaining epithelial cell differentiation and integrity.<sup>14</sup> Retinol deficiency is characterized in the respiratory tract by loss of cilia and squamous metaplasia, changes that are also found in bronchopulmonary dysplasia<sup>10, 14</sup> after neonatal respiratory distress syndrome. At birth, preterm infants have low plasma levels of retinol and retinol-binding protein and possibly insufficient hepatic reserves.<sup>10, 15, 16</sup> Preterm infants who develop bronchopulmonary dysplasia have lower plasma retinol levels than those without this complication.<sup>15, 17</sup>

We chose the primate model to study the effects of

antenatal steroid on serum retinol-binding protein because this model is currently being used to evaluate the effects of dexamethasone on preterm fetal lung maturation.<sup>12</sup> Second, the primate transport system for retinol (vitamin A) has been studied and is nearly identical to that in humans.<sup>1,3</sup>

Our results show that both maternal and fetal serum concentrations of retinol-binding protein increase after antenatal maternal dexamethasone. Fetal levels increased with increasing dexamethasone dosage. This is in agreement with the single earlier report showing an effect of dexamethasone on retinol-binding protein secretion from isolated liver cells in vitro.<sup>9</sup> This is not surprising since steroids are potent regulators of many enzymes and metabolic pathways, and the rapid turnover and metabolic properties of retinol-binding protein make it potentially susceptible to steroid effects. Although we do not have retinol levels, the elevation of retinol-binding protein levels would be expected to result in increased mobilization of retinol from the liver since retinol and retinol-binding protein are usually secreted from the liver bound together in a 1:1 ratio.<sup>4</sup> Early reports suggested that adrenocortical hormones accelerated the release of retinol from the liver.<sup>6,8</sup>

Whether the elevation of serum retinol-binding protein levels, and possibly retinol levels, is a desirable or harmful side effect of antenatal steroid administration is not clear at this time. There are several theoretical reasons why increased retinol-binding protein and retinol levels may be important to the developing lung and offer protection from postnatal injury. Further research is required to determine if antenatal steroids affect the incidence and outcome of respiratory disease in the preterm infant through alterations of retinol and retinol-binding protein concentrations.

The statistical assistance of Mari Palta is appreciated.

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# Human platelet $\alpha$ -adrenergic receptors and responses during pregnancy: No change except that with differing hematocrit

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The possibility that platelet aggregation is altered during pregnancy is controversial. We tested for alterations of  $\alpha$ -adrenergic receptor-induced platelet function during pregnancy compared to that in the early phase of the menstrual cycle. We found no change in  $\alpha$ -adrenergic receptor concentration or in affinity of the receptor for epinephrine. The potency and sensitivity of epinephrine to inhibit adenylate cyclase, a response mediated by  $\alpha_2$ -adrenergic receptors, was not different in platelets from the two groups of subjects. The ability of epinephrine to potentiate aggregation by adenosine diphosphate was significantly increased during pregnancy; however, this was an artifact introduced by lower hematocrits during pregnancy that resulted in an increased citrate concentration in the preparations of platelet-rich plasma from these women. The difference was eliminated by normalization of citrate concentration. Thus effects of  $\alpha$ -adrenergic receptors on platelet function are not different during pregnancy. It is mandatory to recognize artifactual effects of differing hematocrits on platelet aggregation studied in vitro. (AM J OBSTET GYNECOL 1986;154:206-10.)

**Key words:**  $\alpha$ -Adrenergic receptors, platelet aggregation, pregnancy, hematocrit

Pregnancy and especially the postpartum period are associated with an increased risk of thromboembolism.<sup>1</sup> While changes in several components of the coagulation cascade during pregnancy are well established,<sup>1</sup> there is little agreement on the subject of platelet function. In vitro assays have demonstrated that aggregation is increased<sup>2,3</sup> or unchanged<sup>4,5</sup> during pregnancy. This conflicting information can be explained in part by differences in methodology and in part by the use of different agents to initiate aggregation, which may act through different mechanisms.<sup>6</sup> Another problem in interpretation of these studies may be the failure to appreciate the artificial increase in sensitivity of platelet aggregation caused by decreased hematocrit.<sup>7</sup>

We previously reported that treatment of rabbits with estrogen, which decreases platelet aggregation in this species, also decreases platelet  $\alpha$ -adrenergic receptors,<sup>8</sup> which are linked to aggregation. Therefore we decided to examine in detail this pathway for platelet aggregation in human pregnancy to determine if  $\alpha$ -adren-

ergic receptor concentration, agonist affinity for the receptor, and/or linkage of the  $\alpha$ -adrenergic receptor to adenylate cyclase were different during gestation. These results were correlated with the ability of epinephrine to cause aggregation or potentiate adenosine diphosphate-induced platelet aggregation. We report that platelet  $\alpha$ -adrenergic receptor concentration and agonist affinity are similar in pregnant and nonpregnant women as is the ability of epinephrine to inhibit adenylate cyclase. When corrections are made for the decreased hematocrit in pregnant women compared with nonpregnant ones, there is also no change in epinephrine-stimulated platelet aggregation.

## Material and methods

**Materials.** Tritium-labeled yohimbine (specific activity 88 Ci/mmol) and tritium-labeled dihydroergocryptine (specific activity 46 Ci/mmol) were obtained from New England Nuclear. Phentolamine was a gift from Ciba Pharmaceutical Co. and prostaglandin E<sub>1</sub>, a gift from The Upjohn Company. Other materials were the finest quality commercially available.

**Subjects.** Women in the studies were nonsmokers who had taken no medications for 2 weeks and had fasted for at least 3 hours before venipuncture. Pregnant women were between 38 and 40 weeks of gestation, and nonpregnant control subjects were in the first week of the menstrual cycle so that the hormonal environment would be standardized. The follicular phase of the cycle was chosen as a time of low endogenous estrogen levels compared with those of pregnancy since

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animal studies have implicated increased estrogen levels as determinants of platelet adrenergic receptor alterations.

**Preparation of platelet-rich plasma lysates and particulate.** Venous blood was collected through a large-bore needle without stasis into a 1/10 volume of 3.8% sodium citrate and 0.9% sodium chloride (citrate) in plastic syringes. Platelet-rich plasma was prepared by standard techniques.<sup>6</sup> Platelet lysates and particulate were prepared by centrifuging platelet-rich plasma at  $1200 \times g$  for 15 minutes. Platelets were lysed by freeze-thawing. The resulting lysates were diluted and stored at  $-70^\circ\text{C}$  for later use in tritiated dihydroergocryptine-binding assays or centrifuged twice at  $29,000 \times g$  (with an intermediate wash) to be stored and used for adenylate cyclase assays or for binding assays with tritiated yohimbine.

**Aggregation of platelet-rich plasma.** Platelet aggregation was performed on a Payton dual-channel aggregometer.<sup>6</sup> Aggregating agents used were epinephrine ( $10^{-9}$  to  $10^{-5}$  mol/L) dissolved in 1 mmol/L hydrochloric acid and adenosine diphosphate ( $10^{-9}$  to  $10^{-5}$  mol/L) dissolved in double-distilled water. Addition of 25  $\mu\text{l}$  of 1 mmol/L hydrochloric acid had no effect on aggregation. Each aggregating agent was used at several doses to generate a dose-response curve. A new platelet-rich plasma sample was used for each dose of agonist. In addition, a dose-response curve for epinephrine potentiation of adenosine diphosphate aggregation was generated: 60 seconds after addition of 25  $\mu\text{l}$  of epinephrine ( $10^{-9}$  to  $10^{-4}$  mol/L) to platelet-rich plasma, 25  $\mu\text{l}$  of adenosine diphosphate at a concentration sufficient to cause 10% reversible aggregation ( $10^{-7}$  to  $10^{-6}$  mol/L) was added. The time from obtaining of the platelets until their use in the assay and the order of experiments were the same for all samples. In other experiments the citrate concentration in platelet-rich plasma was adjusted to correct for different concentrations of citrate in platelet-rich plasma prepared from blood with different hematocrits.<sup>7</sup>

**Tritiated yohimbine- and tritiated dihydroergocryptine-binding studies.** Studies of tritiated yohimbine and dihydroergocryptine binding were performed at  $30^\circ\text{C}$  in a total volume of 0.25 ml until equilibrium was reached (30 minutes for yohimbine, 60 minutes for dihydroergocryptine). The incubation mixture contained either yohimbine (0.15 to 10 nmol/L) or dihydroergocryptine (1 to 30 nmol/L), platelet particulate or lysates (0.15 to 0.75 mg of protein), 50 mmol/L Tris, pH 7.4, 80 mmol/L sodium chloride, 1 mmol/L ascorbate, and 0.1 mmol/L hydrochloric acid alone or with added adrenergic antagonists. The mixture also contained a final concentration of 2% ethanol to ensure solubility of dihydroergocryptine and yohimbine. This concentration of ethanol had no effect on binding of

either radioligand. Incubations were terminated by the addition of 5 ml of ice-cold wash buffer (50 mmol/L Tris, pH 7.4) and immediate filtration over Whatman GF/C filters under vacuum after three 5 ml washes. Filters were dried by increased vacuum, and radioactivity was counted in a scintillation counter.

Specific binding was defined as the difference between radioactivity bound in the absence of and that bound in the presence of 10  $\mu\text{mol/L}$  phentolamine and composed 75% to 90% of bound radioactivity for yohimbine and 50% to 60% of that for dihydroergocryptine; it was a linear function of added platelet protein over the range of concentrations used in this study. Dissociation constants ( $K_d$ ) for the radioligand and receptor concentration were determined by a computer-assisted analysis of specific bound versus free radioligand.<sup>9</sup> In competition experiments the concentration of competitor that reduced radioligand binding by 50% ( $\text{IC}_{50}$ ) was determined by computer-assisted curve fitting.<sup>9</sup>

Since receptor concentration was low enough to guarantee that free ligand was similar at all competitor concentrations,  $K_i$  could be calculated as:

$$K_i = \frac{\text{IC}_{50}}{1 + L_f/K_d}$$

where  $K_d$  is the dissociation constant for either yohimbine or dihydroergocryptine,  $\text{IC}_{50}$  is the concentration of adrenergic agent required to inhibit specific binding by 50%, and  $L_f$  is the concentration of either yohimbine or dihydroergocryptine in the particular experiment.<sup>10</sup>

**Adenylate cyclase assay.** Assays to determine the activity of adenylate cyclase were performed by a modification of the method of Salomon et al.<sup>11</sup> Platelet particulates (20  $\mu\text{g}$  of protein) were added to incubation tubes containing 50 mmol/L Tris buffer (pH 7.4), 80 mmol/L sodium chloride, 6 mmol/L magnesium chloride, 0.2 mmol/L ethyleneglycol-bis( $\beta$ -aminoethyl ether)-N,N'-tetraacetic acid, 2 mmol/L  $\beta$ -mercaptoethanol, 30 mmol/L guanosine triphosphate, 0.1 mg/ml of bovine serum albumin, 10 mmol/L creatine phosphate, 10 units per milliliter of creatine phosphokinase, 0.4 mmol/L adenosine triphosphate, 1 mmol/L cyclic adenosine monophosphate, and adenosine triphosphate labeled with  $\alpha$ [32-P] (1 to 1.5 million cpm). Tritiated cyclic adenosine monophosphate (approximately 30,000 cpm) was added to determine assay recovery. Some assay tubes also contained 1 mmol/L prostaglandin  $\text{E}_1$ , either alone or in combination with  $10^{-8}$  to  $10^{-3}$  mol/L epinephrine. In addition, each experiment contained one set of tubes that received  $10^{-5}$  mol/L propranolol in conjunction with 1 mmol/L prostaglandin  $\text{E}_1$  and  $10^{-5}$  mol/L epinephrine. The tubes were incubated 10 minutes at  $30^\circ\text{C}$ ; the reaction was stopped,

**Table I.** Platelet aggregation

	Platelet aggregation (mean $\pm$ SEM)			
	Not citrate adjusted		Citrate adjusted	
	Pregnant	Not pregnant	Pregnant	Not pregnant
EC <sub>50</sub> of epinephrine ( $\mu$ mol/L)	1.1 $\pm$ 0.6 (n = 6)	24 $\pm$ 14 (n = 6)	2.4 $\pm$ 0.36 (n = 5)	10.4 $\pm$ 9 (n = 5)
p Value	>0.1*		>0.1*	
Epinephrine plus adenosine diphosphate	0.11 $\pm$ 0.03 (n = 5)	0.34 $\pm$ 0.08 (n = 5)	0.19 $\pm$ 0.09 (n = 5)	0.20 $\pm$ 0.08 (n = 5)
p Value	<0.011†		>0.40†	
Adenosine diphosphate ( $\mu$ mol/L)	1.2 $\pm$ 0.55 (n = 6)	1.6 $\pm$ 0.7 (n = 4)	3.9 $\pm$ 0.9 (n = 6)	2.2 $\pm$ 0.6 (n = 5)
p Value	>0.20†		>0.09†	

\*The p value was determined by Wilcoxon's rank sums. Value is for a one-tailed test.

†The p value was determined by Student's unpaired *t* test. Value is for a one-tailed test.

**Table II.**  $\alpha$ -Adrenergic receptor concentration and dissociation constant

	Pregnant (mean $\pm$ SE)	Nonpregnant (mean $\pm$ SE)
Lysates dihydroergocryptine		
K <sub>d</sub> (nmol/L)	3.4 $\pm$ 0.07 (n = 9)	3.0 $\pm$ 0.3 (n = 12)
B <sub>max</sub> (fmol/mg protein)	178 $\pm$ 27 (n = 9)	174 $\pm$ 20 (n = 12)
Particulate (yohimbine)		
K <sub>d</sub> (nmol/L)	3.2 $\pm$ 2.2 (n = 3)	2.6 $\pm$ 1.1 (n = 3)
B <sub>max</sub> (fmol/mg protein)	196 $\pm$ 8 (n = 5)	228 $\pm$ 28 (n = 5)
Epinephrine K <sub>i</sub> ( $\mu$ mol/L)	4.3 $\pm$ 0.5 (n = 4)	3.3 $\pm$ 0.32 (n = 4)

B<sub>max</sub> = Maximal binding. p > 0.05 for all comparisons.

and cyclic adenosine monophosphate labeled with chromic phosphate P 32 was separated from adenosine triphosphate by sequential Dowex and alumina chromatography.<sup>11</sup> Recovery of cyclic adenosine monophosphate was 75% to 85%.

**Statistical analysis.** If variance was similar between groups comparisons were made by unpaired Student's *t* test. If there was unequal variance a nonparametric comparison (Wilcoxon rank sums) was used. Statistically significant differences were accepted at p < 0.05 for a one-tailed *t* test, since our hypothesis was that platelet aggregation would be more sensitive to epinephrine during pregnancy. Data are presented as the mean  $\pm$  SEM.

## Results

**Aggregation studies.** We initially examined the dose of epinephrine that was needed for half maximal stimulation of platelet aggregation (EC<sub>50</sub>). As has been previously reported, the dose responses were quite steep and it was difficult to accurately determine this parameter.<sup>9,12</sup> However, results suggested an increased sensitivity of platelets of pregnant women to the aggregatory effect of epinephrine (EC<sub>50</sub> = 1  $\pm$  0.6  $\mu$ mol/L in pregnancy and 24.3  $\pm$  14.7  $\mu$ mol/L without pregnancy; p > 0.1). To more accurately and reproducibly determine the sensitivity of platelet aggregation to oc-

cupancy of the  $\alpha$ -adrenergic receptor we examined the ability of epinephrine to augment the aggregation to threshold doses of adenosine diphosphate.<sup>9,17</sup> With this response as the end point, full dose-response curves for  $\alpha$ -adrenergic receptor agonists can be generated. The EC<sub>50</sub> of epinephrine for aggregation studies with platelets from pregnant women (0.11  $\pm$  0.03  $\mu$ mol/L) was significantly less than that with platelets from nonpregnant women (0.34  $\pm$  0.08  $\mu$ mol/L, p < 0.02) (Table I).

Interestingly, dose-response curves for adenosine diphosphate-induced aggregation did not demonstrate a different sensitivity to adenosine diphosphate in pregnancy (EC<sub>50</sub> 1.2  $\pm$  0.20  $\mu$ mol/L in pregnancy and 1.6  $\pm$  0.7  $\mu$ mol/L without pregnancy). The differences in epinephrine sensitivity could not be explained by differences in platelet count. The average count for pregnant women (267,000  $\pm$  71,000/mm<sup>3</sup>) was, as would be predicted, slightly higher than that for nonpregnant women (228,000  $\pm$  87,000/mm<sup>3</sup>). Aggregation differences measured with this technique that were due to different platelet concentrations were not detectable at platelet counts >100,000/mm<sup>3</sup>.<sup>12</sup>

Hematocrit was significantly different in the two groups (35.2%  $\pm$  2.0% in pregnancy and 40%  $\pm$  2.5% without pregnancy). Kelton et al.<sup>7</sup> reported that the sensitivity of the platelet aggregation is artifactually in-

**Table III.** Adenylate cyclase activation

	<i>Pregnant</i>	<i>Nonpregnant</i>
Basal cyclic adenosine monophosphate (pmol/mg/min)	7.4 $\pm$ 1 (n = 5)	6.9 $\pm$ 0.8 (n = 5)
Fold increase with prostaglandin E ( $\mu$ mol/L)	20.8 $\pm$ 1.4 (n = 5)	25.3 $\pm$ 1.7 (n = 5)
Inhibition of prostaglandin E increase by epinephrine (%)	27.6 $\pm$ 2.6 (n = 5)	30.2 $\pm$ 2.6 (n = 5)
EC <sub>50</sub> of epinephrine ( $\mu$ mol/L)	2.7 $\pm$ 0.5 (n = 5)	1.9 $\pm$ 0.4 (n = 5)

p > 0.05 for all comparisons.

creased at low hematocrits because of lower citrate concentrations in platelet-rich plasma. To compensate for differences in citrate concentration in platelet-rich plasma we adjusted the sodium citrate concentration in all samples to that present in blood with a hematocrit of 50 (higher than in any of our subjects) drawn at the usual 1/10 citrate dilution. Blood was drawn at the usual citrate dilution and hematocrit was determined. Sodium citrate was then added to achieve a final volume of citrate determined by the formula:

$$\text{Volume of blood} \times \frac{(1 - \text{hematocrit})}{4.5} = \text{volume of citrate added}$$

After normalization of sodium citrate concentration the difference in epinephrine-induced aggregation was no longer present. The EC<sub>50</sub> for platelet aggregation by epinephrine alone became more similar (2.4  $\pm$  0.36  $\mu$ mol/L in pregnancy and 10.6  $\pm$  9.8  $\mu$ mol/L without pregnancy; p > 0.2). There was no significant difference in the EC<sub>50</sub> of epinephrine to potentiate adenosine diphosphate-induced aggregation (0.19  $\pm$  0.09  $\mu$ mol/L in pregnancy and 0.20  $\pm$  0.08  $\mu$ mol/L without pregnancy; p = 0.46) (Table I).

**Radioligand binding studies.** With dihydroergocryptine used as the radioligand there was no difference in  $\alpha$ -adrenergic receptor concentration in lysates prepared from platelets of pregnant (178  $\pm$  82 fmol/mg of protein) and nonpregnant (174  $\pm$  46 fmol/mg of protein) women. There was no difference in the K<sub>d</sub> of dihydroergocryptine (Table II).

Since dihydroergocryptine binds to sites other than  $\alpha$ -adrenergic receptors and nonselectively to both  $\alpha_1$ - and  $\alpha_2$ -adrenergic receptors,<sup>13-15</sup> an increase in  $\alpha_2$ -adrenergic receptors might be masked by a decrease of either  $\alpha_1$ -adrenergic receptors or other unknown sites. Therefore we also examined the binding of yohimbine, an  $\alpha$ -adrenergic receptor antagonist that binds selectively and with high affinity to  $\alpha_2$ -adrenergic receptors.<sup>13</sup> In our initial experiments receptor concentration was determined in lysates because we were concerned that recovery of platelet particulate might be influenced by pregnancy. To eliminate the possibility that proteolysis might be greater in platelet lysates of pregnant women and thus falsely lower receptor concentration, we examined binding of yohimbine to washed platelet

particulates. Because preliminary experiments indicated no difference in the K<sub>d</sub> of yohimbine for platelets during pregnancy (K<sub>d</sub> = 3.2  $\pm$  2.2 in pregnancy and 2.6  $\pm$  1.1 nmol/L without pregnancy), we used a saturating concentration of yohimbine (24 nmol/L) with 10<sup>-5</sup> mol/L phentolamine to determine nonspecific binding, for estimation of receptor concentration. The estimate of receptor concentration by this method demonstrated no difference between platelets of pregnant (196  $\pm$  20 fmol/mg of protein) and nonpregnant (228  $\pm$  63 fmol/mg of protein) women (Table II). In addition, experiments examining the ability of epinephrine to compete for yohimbine binding to platelet particulation showed that the affinity of epinephrine for the  $\alpha_2$ -adrenergic receptor was not different in platelets from pregnant and nonpregnant women (K<sub>i</sub> 4.3  $\pm$  0.3  $\mu$ mol/L in pregnancy and 3.3  $\pm$  0.32  $\mu$ mol/L without pregnancy).

**Adenylate cyclase inhibition.** To probe the linkage of  $\alpha_2$ -adrenergic receptor occupancy to platelet aggregation we examined the ability of epinephrine to inhibit adenylate cyclase<sup>15</sup> that has been stimulated by prostaglandin E in platelet particulate from pregnant and nonpregnant women. Basal and prostaglandin E-stimulated adenylate cyclase activity was similar in both groups (Table III). In addition neither the EC<sub>50</sub> of epinephrine (2.7  $\pm$  0.5  $\mu$ mol/L in pregnancy and 1.9  $\pm$  0.4  $\mu$ mol/L without pregnancy) nor the maximum decrease of adenylate cyclase by epinephrine (27.6%  $\pm$  2.6% in pregnancy and 30.2%  $\pm$  2.6% without pregnancy) was different (Table III). Addition of propranolol did not potentiate the effect of epinephrine (data not shown).

### Comment

Our initial examination of platelet aggregation without correction for hematocrit supported our hypothesis that platelet  $\alpha$ -adrenergic receptor response and perhaps number of receptors were affected by estrogen as previously described for these receptors in rabbits.<sup>8, 16</sup> There was an increased sensitivity to epinephrine, determined as the EC<sub>50</sub> of epinephrine to augment adenosine diphosphate response, without a generalized increased sensitivity since adenosine diphosphate alone induced aggregation with a similar sensitivity in both groups.

We were therefore surprised to find that the platelet  $\alpha$ -adrenergic receptor concentrations of pregnant and nonpregnant women were not different. This was the case with two  $\alpha$ -adrenergic radioligands and whether determined in lysates or particulates. There was also no change in the affinity of epinephrine for  $\alpha_2$ -adrenergic receptors.

The linkage of  $\alpha_2$ -adrenergic receptor occupancy to subsequent response also did not appear different since adenylate cyclase inhibition by epinephrine was also similar in the two groups. We could not reconcile these similarities in  $\alpha$ -adrenergic receptor concentration and function with our original finding of an increase in epinephrine-induced platelet aggregation in pregnancy. Since platelet function in vitro may be influenced by several factors, we reexamined our assay techniques.

Platelet aggregation in vitro is altered by many subtle variations in methodology. The time from platelet removal to assay can alter responses. In all of our experiments we carefully controlled time from drawing of blood to assay and the order of experiments. Platelet count affects platelet aggregation but only at platelet concentrations substantially less than were present in these subjects.<sup>12</sup>

Kelton et al.<sup>7</sup> have pointed out the importance of adjusting the sodium citrate concentration in platelet-rich plasma to eliminate differences brought about by use of a fixed concentration of sodium citrate with blood samples with different hematocrits. Sodium citrate does not enter cells, thus lower hematocrit results in a lower concentration of sodium citrate in platelet-rich plasma. Since sodium citrate is a calcium chelator, platelet-rich plasma from blood samples with lower hematocrits (and higher free calcium concentrations), as in our samples from pregnant women, will have enhanced aggregation. Interestingly, in the study of Kelton et al. as in our own, the influence is greater on epinephrine than on adenosine diphosphate-induced aggregation. We found that the elimination of differences in sodium citrate concentration eliminated differences of epinephrine sensitivity of platelets. Thus similar  $\alpha$ -adrenergic receptor concentrations, agonist affinity, and coupling to adenylate cyclase in the two groups are compatible with the similar sensitivity to epinephrine when the confounding variable of a different sodium citrate concentration is controlled.

This variable was not controlled in any of the previous comparisons of platelet aggregation in pregnant and nonpregnant women,<sup>2-5</sup> which accounts in part for

the conflicting results. Kelton et al.<sup>7</sup> pointed out that many reported differences in platelet aggregation between sexes and with disease are invalidated by failure to correct for different hematocrits. This confounding variable must be taken into account when the differences in platelet aggregation are reported in pathologic pregnancies.

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## Severe hypoglycemia associated with the HELLP syndrome

To the Editors:

We read with interest the case report of Egley et al. entitled "Severe hypoglycemia associated with HELLP syndrome" (*AM J OBSTET GYNECOL* 1985;152:576). The initial presentation was not typical of the HELLP syndrome, however. A review of most cases in the literature<sup>1-3</sup> and our own experience with 110 cases indicate that the platelet count is usually low and the fibrinogen, protime, and partial thromboplastin time usually normal on presentation. In this case the platelet count was 156,000/mm<sup>3</sup> and fibrinogen was 37 mg/dl. The initial protime and partial thromboplastin time were not reported. We feel that the more likely diagnosis is acute fatty liver of pregnancy.

Patients with acute fatty liver characteristically present in the third trimester with a 2- to 3-week history of nausea, vomiting, epigastric pain, jaundice, and fetal death—as this patient did. Laboratory results include mild to marked leukocytosis, a serum glutamic oxaloacetic transaminase level of <500 µ/ml, mild increase in alkaline phosphatase, hyperbilirubinemia, hypoprothrombinemia, marked hyperuricemia, hypoglycemia, and elevated blood urea nitrogen, creatinine, and blood ammonia levels. Features suggesting preeclampsia are present in 20% to 40% of cases. All these findings are consistent with the case reported. The patient may die of hepatic failure or succumb to extrahepatic manifestations such as renal failure, hemorrhagic pancreatitis, disseminated intravascular coagulation, sepsis, or shock. The early literature indicated a dismal prognosis with 75% to 85% maternal and 85% to 90% fetal mortality. Recent reports<sup>1-3</sup> indicate that the mortality from the illness has dropped. Because of better supportive care and recognition by liver biopsy of milder forms of the disease, it has been found that many patients improve rapidly 1 to 2 weeks after delivery.

We agree with the authors that the HELLP syndrome is only one point on a spectrum of hematologic and liver involvement in preeclampsia. Furthermore, we realize that there may be considerable overlap in the laboratory data in HELLP syndrome and acute fatty liver and that liver biopsy would be necessary in this case to prove the diagnosis of acute fatty liver. Finally, we would be interested in knowing some additional laboratory data on admission (white blood cell count, blood urea nitrogen, creatinine, protime, and partial thromboplastin time) and the length of time until the liver function tests returned to normal.

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## Reply

To the Editors:

We wish to thank Mabie and Sibai for their interest in our paper entitled "Severe hypoglycemia associated with HELLP syndrome." The purpose of our paper was to note that hypoglycemia can occur as part of the HELLP syndrome and that the hypoglycemia might be avoided through intravenous dextrose infusion. The purpose was not to argue the semantics of "HELLP syndrome" versus "acute fatty liver of pregnancy." In fact, the HELLP syndrome is just that—a syndrome. The diagnosis of acute fatty liver of pregnancy, on the other hand, requires histologic evaluation of the liver.

The patient described in our paper clearly had HELLP syndrome. Hemolysis was present (fall in hematocrit, schistocytes present on peripheral smear), liver enzyme level was elevated, and the platelet count was low (109,000/mm<sup>3</sup>). The letter implies that the initial platelet count (156,000/mm<sup>3</sup>) was normal. However, this value is low even for the third trimester of pregnancy<sup>1</sup> and is clearly lower than that in several patients reported with HELLP syndrome.<sup>2</sup>

The initial protime (20.7 seconds) and partial thromboplastin time (54.3 seconds) were slightly prolonged. Three of the original five patients with HELLP syndrome had prolonged partial thromboplastin time values.<sup>3</sup> In our patient these values were certainly consistent with the marked depression in the levels of hepatically produced coagulation factors.

The case of HELLP syndrome presented in our paper may have been associated with acute fatty liver of pregnancy. The diagnosis could only have been made through liver biopsy, which we strongly considered. Liver biopsy was contraindicated early in the course of the illness because of coagulopathy. After the patient's rapid recovery we felt that a liver biopsy was not justified because the results of the biopsy would have been

of academic interest only and would not have altered the treatment. Several clinical and laboratory findings, however, would dispute the diagnosis of acute fatty liver of pregnancy: (1) the clinical onset was not abrupt; (2) the patient was afebrile; (3) rapid recovery of renal function (blood urea nitrogen, 7 mg/dl; creatinine, 1 mg/dl) by 24 hours after delivery; (4) protime was never greater than 20.7 seconds; (5) maximum white count was 18,000/mm<sup>3</sup>; (6) total bilirubin began to fall the day after delivery; (7) the liver was not palpably enlarged.

Finally, we are happy to supply the additional admission laboratory values requested: admission blood urea nitrogen, 22 mg/dl; creatinine 3.2 mg/dl; and admission white count, 18,600/mm<sup>3</sup>. All liver function tests had returned to normal by 7 days after delivery.

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#### Placental prostacyclin production in normal and toxemic pregnancies

To the Editors:

The study by Walsh et al. (Placental prostacyclin production in normal and toxemic pregnancies. *AM J OBSTET GYNECOL* 1985;151:110-15) showing diminished prostacyclin production by placental tissue obtained from patients with toxemia is of great interest. A few additional comments may also be of value.

Others<sup>1</sup> have also investigated placental 6-oxo-prostaglandin F<sub>1α</sub> production in hypertensive pregnancies. They reported comparable amounts of 6-oxo-prostaglandin F<sub>1α</sub> released by healthy placentas but found no differences in production of this prostanoid in hypertensive pregnancies.<sup>1</sup> They did, however, find that production of thromboxane A<sub>2</sub> (a vasoconstrictor and promoter of platelet aggregation) was increased in placentas obtained from hypertensive pregnancies.<sup>1</sup>

The production rate of prostacyclin reported by Walsh et al. in healthy placental tissue (7.2 pg · mg<sup>-1</sup> · hr<sup>-1</sup>) is also comparable to our finding<sup>2</sup> of 12 pg · mg<sup>-1</sup> · hr<sup>-1</sup>. However, it is worth considering these rates in the context of synthesis by other organs; for example, prostacyclin production by umbilical arteries and veins is 7.7 and 3.8 ng · mg<sup>-1</sup> · hr<sup>-1</sup>, respectively.<sup>2</sup> Therefore these vessels released several hundredfold

more prostacyclin per unit wet weight than the placentas obtained from the same healthy subjects.<sup>2</sup> This difference in production rates does not exclude a biologically relevant role for prostacyclin in the placenta, but it does suggest that other protective mechanisms operate to maintain the patency of this vascular organ. In this context we have reported<sup>3-6</sup> the presence, in placental tissue, of an active "adenosine diphosphatase" system that converts proaggregatory adenosine diphosphate into adenosine, a vasodilator and potent inhibitor of platelet aggregation. Although the *in vivo* role of placental prostacyclin and adenosine diphosphatase remains difficult to define, there is little doubt that the platelet antiaggregatory activity of the placenta *in vitro* must be ascribed to adenosine diphosphatase.<sup>7</sup> It would therefore be of interest to determine whether toxemia of pregnancy affects placental adenosine diphosphatase production.

The relatively low production rate of prostacyclin by placental tissue also suggests that comments regarding the contribution of this organ to systemic levels of prostacyclin must be guarded. This cautionary note is further reinforced by two additional findings: (1) in healthy, nonpregnant subjects, circulating levels of 6-oxo-prostaglandin F<sub>1α</sub>, the stable spontaneous breakdown product of prostacyclin, are very low or even undetectable when measured by what must be the reference method (gas chromatography-mass spectrometry)<sup>8</sup>; (2) prostacyclin release from umbilical vessels has been shown to be diminished in toxemia (as cited by Walsh et al.). It follows that even if there were substantial amounts of circulating prostacyclin (measured by the method of choice) during pregnancy, these levels as well as their pathologic changes are more likely to reflect altered vessel prostacyclin synthesis than what appears to be only minimal placental release of this prostanoid.

Urine prostanoid levels were also cited by Walsh et al. However, before these levels are considered to reflect systemic and/or renal prostanoid production, it should be noted that the urinary bladder of the rat,<sup>9</sup> the cat,<sup>10</sup> and the guinea pig (unpublished observations) produces substantial amounts of prostacyclin, the synthesis of which is influenced by several variables,<sup>9, 10</sup> including pH, osmolarity, distention, cigarette smoke extracts, and the presence of diabetes (unpublished observation).

We have also demonstrated<sup>2</sup> that indomethacin inhibits placental prostacyclin production and that arachidonic acid is not substantially converted to prostacyclin by placental tissue. However, we do not agree that the latter phenomenon relates to adequate or inadequate endogenous arachidonic acid (i.e., precursor) availability in placental tissue, since (1) human placental tissue fragments, homogenates, and cytosol and microsomal preparations cannot convert endoperoxides into prostacyclin,<sup>11</sup> and (2) microsomes from rabbit placentas generate little or no prostacyclin.<sup>12</sup> Therefore the most likely explanation is that the capacity of placental

tissue to convert arachidonic acid into prostacyclin is limited, regardless of precursor availability. We have also established<sup>2</sup> that placental extracts did not inhibit prostacyclin synthesis by umbilical vessels. Finally, it is relevant to describe the area of placenta sampled, since we have shown<sup>7</sup> that large vessels entering the placenta produce prostacyclin.

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#### Reply

To the Editors:

We are pleased that Dr. Jeremy and colleagues found our paper on decreased prostacyclin production by toxemic placentas to be of great interest and took the time to comment and expand on the data. It is comforting to see that their prostacyclin production rate for normal placentas agrees so closely with ours.

We are aware that prostacyclin production rates by

umbilical vessels are severalfold higher than by whole placental tissues when expressed on a per milligram basis. This does not diminish the potential role of placently produced prostacyclin to locally inhibit platelet aggregation and maintain placental vasodilatation. Comparison of production rates on a per milligram whole tissue basis between a purely vascular tissue and the placenta is misleading. It must be remembered that the placenta is composed of several different tissues, including trophoblast, stroma, and vascular tissue. Prostacyclin is likely produced primarily by the endothelial cells of the placental vessels with the trophoblast perhaps contributing some. Although the prostacyclin production rate is small when expressed in milligram of wet tissue per hour, the large placental mass makes it a formidable endocrine organ during pregnancy. Our production rate of 7.2 pg/mg/hr is equivalent to approximately 94 µg per placenta/per day. This is certainly a high enough production rate to produce physiologic effects and to affect fetal and/or maternal circulating levels; provided the placenta or lungs do not first bind or metabolize the prostacyclin. Although recent studies with use of gas chromatography-mass spectrometry question whether prostacyclin is a circulating hormone in nonpregnant individuals,<sup>1</sup> there is considerable controversy in the literature regarding this question and the circulating concentrations of prostacyclin.<sup>2-9</sup>

The presence of an active placental "adenosine diphosphatase" system that converts proaggregatory adenosine diphosphate into adenosine is interesting and probably involved in placental vasodilation and inhibition of platelet aggregation. Prostacyclin, however, is 1000 times more potent than adenosine in inhibiting platelet aggregation,<sup>10</sup> so the placental production rate of adenosine would have to be considerably greater than that of prostacyclin to be equipotent. Placental tissue levels of adenosine have been measured,<sup>11</sup> but we are unaware that the placental production rate of adenosine has been determined so it is difficult at this time to compare its physiologic importance with prostacyclin. Furthermore, it must be determined whether adenosine is a vasodilator in the human placenta because adenosine does not vasodilate all vascular beds. For example, adenosine vasoconstricts the renal glomerular vascular bed<sup>12,13</sup> and the sheep fetal placental vascular bed.<sup>13</sup> Prostacyclin, on the other hand, maintains human placental vasodilation in the face of an angiotensin II vasoconstrictor challenge.<sup>14</sup>

We agree that placental prostacyclin production is not influenced by precursor availability. The data presented in the paper suggest that prostacyclin production is limited by the amount of enzyme present, not the amount of arachidonic acid. We also agree that prostacyclin production can vary in different areas of the placenta. We controlled for this by taking tissue from various sites and always from the interior of the placenta to exclude contamination with chorion, decidua, or large blood vessels.

Hemodynamic regulation within the placenta is undoubtedly complex, involving synergistic and antagonistic actions of various chemical mediators such as prostacyclin, thromboxane, adenosine, angiotensin, catecholamines. We recently reported that preeclampsia is characterized by a placental imbalance of increased thromboxane and decreased prostacyclin production.<sup>15</sup> The normal placenta produced equal amounts of the two eicosanoids so their biologic actions would be balanced. The preeclamptic placenta, however, produced over seven times as much thromboxane as prostacyclin so thromboxane's actions to promote vasoconstriction, platelet aggregation, and uterine contractions would predominate. There are further abnormalities in arachidonic acid metabolism in the preeclamptic placenta. We also recently reported that placental 5-hydroxyeicosatetraenoic acid and 12-hydroxyeicosatetraenoic acid production rates are significantly reduced in preeclampsia indicating aberrations in the lipoxygenase pathways.<sup>16</sup> Furthermore, 5-hydroxyeicosatetraenoic acid inhibits thromboxane and prostacyclin production, and cyclooxygenase products inhibit lipoxygenase product production and increase their metabolism.<sup>17</sup> Therefore, there appears to be short loop feedback systems between the cyclooxygenase and lipoxygenase metabolites within the placenta. There is obviously much to be learned about the hemodynamic control mechanisms of the human placenta and their aberrancies in disease, the knowledge of which is a necessary prerequisite to the safe, effective treatment of clinical disorders such as preeclampsia.

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#### Percutaneous umbilical sampling in immune thrombocytopenic purpura

To the Editors:

In their article, entitled "Percutaneous umbilical blood sampling" (*AM J OBSTET GYNECOL* 1985;152:1), Dr. Hobbins and his colleagues suggest applying this technique to cases of immune thrombocytopenic purpura. I would be interested in their comments about a few related issues.

The authors indicated that they distinguish maternal from fetal red blood cells with an electronic cell analyzer and a Kleihauer-Betke test. Do they have a method for distinguishing maternal from fetal platelets?

O'Reilly and Taber<sup>1</sup> and Territo et al.<sup>2</sup> suggested that fetuses at greatest risk of thrombocytopenia were those whose mothers were thrombocytopenic. If the mother is thrombocytopenic, do the authors give platelet transfusions just before percutaneous sampling?

Clinical evidence is lacking that routine cesarean section before the onset of labor prevents intracranial hemorrhage in thrombocytopenic infants of mothers who have immune thrombocytopenic purpura. Because of either the infrequency of the disease or a failure to recognize it in an otherwise asymptomatic pa-



tient, resolution of the issue likely awaits the execution of a randomized, controlled, multi-institutional study. A spectrum of opinion exists ranging from delivering all infants by cesarean section<sup>3</sup> to performing cesarean section only in cases of thrombocytopenic mothers<sup>2</sup> to performing cesarean section for obstetric indications only.<sup>1,4</sup> Given the controversy, a test that purports to assist in deciding the route of delivery should carry with it minimal risk. Bleeding from a fetal scalp sampling site, even in a thrombocytopenic fetus, may be controlled by direct pressure.<sup>5</sup> However, bleeding from an umbilical cord puncture site may not be so controlled. The authors reported two cases of immune thrombocytopenic purpura, in both of which the fetal platelet count exceeded 200,000/mm<sup>3</sup>. I would appreciate their sharing their experience, if any, in performing percutaneous umbilical sampling in fetuses found to be thrombocytopenic.

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#### Reply

To the Editors:

We appreciate Dr. Sacks' interest in our work. He raised a number of provocative questions.

First, he asked whether we have a method for distinguishing maternal from fetal platelets. We do not. His question implies the possibility of maternal platelets crossing the placental barrier and causing a false elevation of the fetal platelet count. We are unaware of any evidence or precedence for such a phenomenon. Indeed, Wallenburg et al.<sup>1</sup> have reported the inability of chromium 51-radiolabeled maternal platelets to cross the placental barrier in the rhesus monkey. Since our platelet counts are measured in specimens containing only fetal red blood cells, maternal contamination is quite unlikely.

Second, he asked whether we have given platelet transfusions to thrombocytopenic mothers before per-

cutaneous umbilical cord sampling. Thus far we have not. Our patients have all had platelet counts >60,000/mm and/or a normal bleeding time. The bleeding time has been used to test the competence of the platelet component of the hemostatic system. In the presence of a prolonged bleeding time, we would transfuse platelets. They would be indicated to protect the mother from morbidity associated with hemostatic failure.

Finally, Dr. Sacks inquired as to the safety of percutaneous umbilical cord puncture in the thrombocytopenic fetus. Since all three of our fetuses had normal platelet counts, we do not have data to answer his question. However, it should be pointed out that percutaneous umbilical cord puncture with a 20-gauge needle was successfully used by Daffos and Forester<sup>2</sup> to transfuse in utero a thrombocytopenic fetus without complications. Of course, this does not constitute absolute proof that a thrombocytopenic fetus will not bleed when the procedure is performed with a 25-gauge needle. For this reason we perform our procedure on the delivery floor and have the patient prepared for a possible cesarean section.

Dr. Sacks justly mentioned the controversy surrounding the optimal mode of delivery for the mother with immune thrombocytopenic purpura. Our article describes a procedure that allows us to obtain information about platelet counts before the onset of labor or when dilatation is insufficient to allow a scalp platelet count. The advantages of this procedure over the standard approach of scalp platelet sampling remain to be established. The intent of our article was to describe a technique that we think has far-reaching potential.

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#### Treatment of endometriosis

To the Editors:

Operation for endometriosis is based on the assumption that the disease is a surface phenomenon that can be seen, diagnosed, and treated by laparoscopy or laparotomy and that the adhesions and other lesions can be eradicated by scalpel, Bovie, or laser. Unfortunately, the peritoneal adhesions contain living, active

endometriotic cells that "menstruate" with each menstrual cycle and cause progressive symptoms. It is not enough to "reduce" these adhesions, as a surgeon would reduce inflammatory adhesions, and if the surgeon attempts to eradicate the adhesions completely, there is risk of perforation of bladder or gut. Endometriosis is deeply implanted in the uterus, salpinx, and ovary where it cannot be seen or treated surgically. Hysterectomy-oophorectomy is the best the surgeon can offer, and this operation in a "frozen" pelvis is very difficult and hazardous. The disease must be treated by systemic, endocrine methods.

Progesterone, testosterone, danazol, and gonadotropin-releasing hormone have all failed to eradicate endometriotic cells, and each has undesirable side effects. Only diethylstilbestrol, properly given, can diagnose the disease without invasion, eradicate endometriotic cells, and safely preserve fertility and femininity. There is a prejudice against diethylstilbestrol in the minds of most gynecologists that is not justified by fact. It is a synthetic, very powerful estrogen (600 times as strong as ethinyl estradiol, another synthetic estrogen). It is this powerful, estrogen receptor-binding action of diethylstilbestrol that makes the drug more effective than other, weaker estrogens or other drugs. Diethylstilbestrol is not hepatotoxic even in astronomic dosage. It has never been proved to be the cause of any malignancy although it has been "associated with" various malignancies. Its use in treating endometriosis in premenopausal women is without any cancer risk, as shown by its use in more than 5000 cases of endometriosis (Karnaky KJ, unpublished observations). All estrogens stimulate "target" cells that have already become malignant, and this is the basis of the "associated with" indictment. Clear cell carcinoma of the vaginal vault is a rare, embryogenic neoplasm of low malignancy that nature was attempting to dispose of in the first trimester, but diethylstilbestrol was given to stop the abortion and thus became "associated with" the malignancy. In one third of the cases there is no history of diethylstilbestrol given the mother, and now more and more cases not associated with the drug are being found.<sup>2</sup>

Use of diethylstilbestrol in treating endometriosis is simple and without hazard. Pregnancy must be ruled out. The dosage must be very low at the beginning to avoid nausea. The total dosage is 50 mg/day for 90 days. Bleeding in the latter days is easily controlled by suction curettage or with progesterone for the few remaining days of treatment. The patient feels well and accepts the treatment even if a repeat course becomes necessary. The patient says, "I have never felt better in my adult life! Can't I just continue taking the drug indefinitely?"

W. E. Lockhart, Jr., M.D.  
Karl John Karnaky, M.D.

401 N. 4th Street  
Alpine, Texas 79830

## REFERENCE

1. International Medical News Service, August, 1985.

## Proper study and use of reference material

### To the Editors:

The appropriate use of reference material can enhance the quality of a scientific manuscript. However, careless use of the same may have the opposite effect. Gortmaker et al. (Gortmaker S, Sobol A, Clark C, Walker DK, Geronimus A. The survival of very low-birth weight infants by level of hospital of birth: A population study of perinatal systems in four states. *AM J OBSTET GYNECOL* 1985;152:517) documented the value of properly functioning regional systems of perinatal health care or care matched with need. Unfortunately, they incorrectly used a reference to my work to embellish a point.<sup>1</sup>

Specifically, they stated: "In Iowa, in 1978, only 22% of very low-birth weight infants were born in that state's one regional center." They cited my work as an example of the lack of successful development of regionalized perinatal systems.

The authors would have done well to have read the entire referenced article, for indeed my work makes the same basic point and is not contrary evidence as noted.

In 1978, 22% of all very low birth weight neonates in Iowa were born at The University of Iowa Hospitals and an additional 35.6% were born at 10 regional centers. My article also shows that only 33.6% of births of very low birth weight infants occurred in the 11 center hospitals in 1972 whereas 58.2% of such births occurred in these hospitals in 1978. The 11 regional facilities accounted for 27.0% of all births in 1972 and 34.3% of all births in 1978. Accordingly, I believe the referenced data clearly show that, in 1978, births of high-risk (very low birth weight) infants were occurring much more frequently in the centers and hence the desired effect of a regionalized system of care was being realized.

Iowa and other rural states will never have the luxury of geographically apportioned tertiary level facilities comparable to major medical centers such as The University of Iowa Hospitals. However, our modification of the traditional system serves this state well and currently (1983 data from the Iowa State Health Department) 77.7% of births of all very low birth weight infants occur at The University of Iowa Hospitals and the regional centers.

The work of Gortmaker et al. carries an important message. More precise attention to reference sources would have enhanced that message.

Herman A. Hein, M.D.

Department of Pediatrics  
The University of Iowa Hospitals and Clinics  
Iowa City, Iowa 52242

#### REFERENCE

1. Hein H. Evaluation of a rural perinatal care system. *Pediatrics* 1980;66:640.

#### Reply

*To the Editors:*

We regret that our reference to the Iowa experience was not more complete. As Hein correctly noted, regionalization in Iowa has proceeded successfully with the increasing utilization of Level II as well as Level III centers. One lingering issue, however, is the extent to which very low birth weight infants born in Level II centers experience greater mortality compared with that in similar infants born in tertiary centers. This was the case indicated for Iowa (see Table 7 of Hein's reference), although the differences were not statistically significant. This situation has also been reported for New York City, where Paneth et al.<sup>1</sup> found evidence of

greater mortality among some very low birth weight infants born in Level II centers when compared with that in similar infants born in Level I as well as Level III centers. Thus continuing evaluation of differential mortality, depending upon the level of hospital of birth, is required as different models of regionalization proceed.

*Steven L. Gortmaker, Ph.D.*

*Department of Behavioral Sciences  
School of Public Health  
Harvard University  
677 Huntington Avenue  
Boston, Massachusetts 02115*

#### REFERENCE

1. Paneth N, Kiely JL, Susser M. Age at death used to assess the effect of interhospital transfer of newborns. *Pediatrics* 1984;73:854.

#### Erratum

In the September 1, 1985, issue of the JOURNAL, in the article entitled "Alloimmunization in twin pregnancies," by J. M. Bowman, M.D., in Table IV, which appears on pages 10 and 11, under the heading Comments, the statement regarding the ninth set of twins is incorrect. The statement should have been: "Twin B, group A compatible, was less ill twin."

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(1945) *President*, Morrie M. Gelfand. *Secretary*, Patrick T. Mohide, McMaster University, Faculty of Health Sciences, Department of Obstetrics and Gynecology, 1200 Main St. West, Room 407, Hamilton, Ontario, Canada L8N 3Z5. Meeting, June 23-27, 1986.

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24, Downstate Medical Center, Brooklyn NY 11203. Meetings, third Wednesday of Jan., Feb., March, April, and May.

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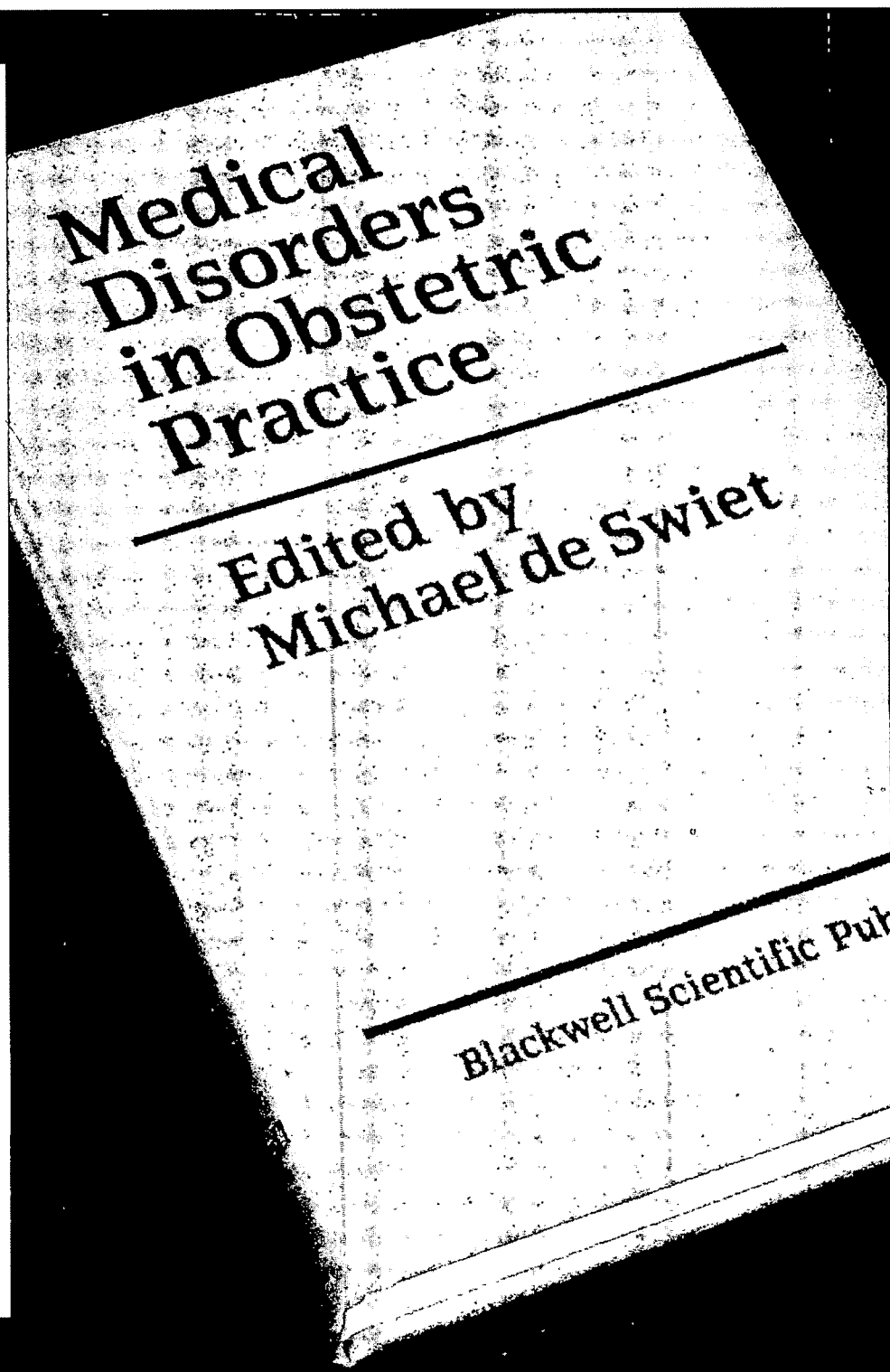
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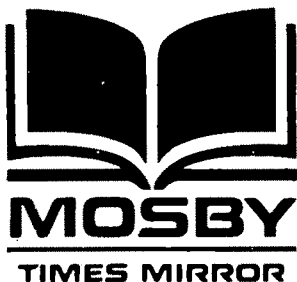
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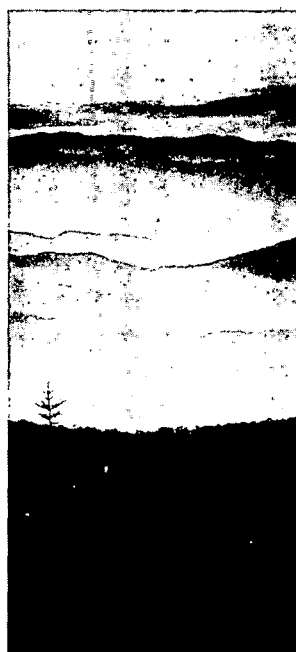
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**CONTRAINDICATIONS:** Oral contraceptives should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophlebitis or thromboembolic disorders. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Oral contraceptive tablets may cause fetal harm when administered to a pregnant woman. Oral contraceptive tablets are contraindicated in women who are pregnant. If the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus (see WARNINGS, No. 5). 8. Benign or malignant liver tumor which developed during the use of oral contraceptives or other estrogen-containing products.

### WARNINGS

**Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy cigarette smoking and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.**

**The use of oral contraceptives is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.**

1. **THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS.** An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Four principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both of which are of the deep vein thrombotic type. The studies estimate that users of oral contraceptives are 4 to 11 times more likely than nonusers to develop these diseases without evident cause. Overall excess mortality due to pulmonary embolism or stroke is on the order of 10 to 35 deaths annually per 100,000 users and increases with age. **CEREBROVASCULAR DISORDERS:** In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than in nonusers and the risk of thrombotic stroke was 4.0 to 9.5 times greater in users than in nonusers. A prospective study conducted in Great Britain estimated that former users have a risk for all cerebrovascular disease 2.6 times greater than that of nonusers. This risk remained elevated for at least six years after last oral contraceptive use. A prospective study conducted in the United States found that past use of oral contraceptives was associated with increased risk of subarachnoid hemorrhage, the relative risk being 5.3. There was also some evidence from this study that the degree of risk may be related to duration of oral contraceptive use. **MYOCARDIAL INFARCTION:** An increased risk of myocardial infarction associated with the use of oral contraceptives has been reported in a previously suspected association. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preclampsia toxemia), the higher the risk of developing myocardial infarction, regardless of whether the patient was an oral contraceptive user or not. Oral contraceptives, however, were found to be a clear additional risk factor. The annual excess case rate (increased risk) of myocardial infarction (fatal and nonfatal) in oral contraceptive users was estimated to be approximately 7 cases per 100,000 women users in the 30-39 age group and 67 cases per 100,000 women users in the 40-44 age group. In terms of relative risk, it has been estimated that oral contraceptive users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. Oral contraceptive users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified, it is quite likely that the same synergistic action exists, but perhaps to a lesser extent. A study suggests that some increased risk of myocardial infarction in oral contraceptive users persists following discontinuation of oral contraceptives and that the degree of the residual risk is related to the duration of the past use. **Risk of Dose:** In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in oral contraceptives. Preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the United States. Careful epidemiological studies to determine the degree of thromboembolic risk associated with progestogen-only oral contraceptives have not been performed. Cases of thromboembolic disease have been reported in women using these products, and they should not be presumed to be free of excess risk. The risk of thromboembolic and thrombotic disorders, in both users and nonusers of oral contraceptives, increases with age. Oral contraceptives are, however, an independent risk factor for these events. **ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES:** A large prospective study carried out in the United Kingdom estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of oral contraceptives according to age, smoking habits, and duration of use. The overall excess death rate annually from circulatory diseases for oral contraceptive users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), the risk being concentrated in older women, in those with a long duration of use, and in cigarette smokers. It was not possible, however, to examine the interrelationships of age, smoking, and duration of use, nor to compare the effects of continuous versus intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for five or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for five or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. This study reports that the increased risk of circulatory disease mortality may persist after the pill is discontinued. Another study published at the same time confirms a previously reported increase of mortality in pill users from cardiovascular disease. The study concluded that the mortality associated with all methods of birth control is low and that that associated with childbirth, with the exception of oral contraceptives in women over 40 who smoke. (The rates given for pill only smokers for each age group are for smokers as a class. For "heavy" smokers [more than 15 cigarettes a day], the rates given would be about double; for "light" smokers [less than 15 cigarettes a day], about 50 percent.) The mortality associated with oral contraceptive use in nonsmokers over 40 is higher than with any other method of contraception in that age group. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with oral contraceptives increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of preclampsia toxemia and especially by cigarette smoking. The risk of myocardial infarction in oral contraceptive users is substantially increased in women age 40 and over, especially those with other risk factors. The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four- to six-fold increased risk of postsurgery thromboembolic complications has been reported in oral contraceptive users. If feasible, oral contraceptives should be discontinued at least four weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization. 2. **OCULAR LESIONS:** There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of oral contraceptives. Discontinue oral contraceptive use if there is unexplained, sudden or gradual, partial or complete loss of vision, onset of proptosis or diplopia, papilledema, or retinal vascular lesions and institute appropriate diagnostic and therapeutic measures. 3. **CARCINOMA:** Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in oral contraceptives, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case control studies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on oral contraceptives. Of the cases found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time oral contraceptives were first given, polycystic ovaries), nearly all occurred in women who had used a sequential oral contraceptive. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only oral contraceptives. Several studies have found no increases in breast cancer in women taking oral contraceptives or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with oral contraceptives, found an excess risk in the subgroups of oral contraceptive users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of oral contraceptives has been well-documented. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with oral contraceptives. Close clinical surveillance of all women taking oral contraceptives is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. 4. **HEPATIC TUMORS:** Benign hepatic adenomas have been found to be associated with the use of oral contraceptives. One study showed that oral

# Low breakthrough bleeding

Tablets

contraceptive formulations with high hormonal potency were associated with a higher risk than lower potency formulations and use of oral contraceptives with high hormonal potency and age over 30 years may further increase the woman's risk of hepatocellular adenoma. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of oral contraceptives. Two studies relate risk with duration of use of the contraceptive, the risk being much greater after four or more years of oral contraceptive use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women taking oral contraceptives. The relationship of these drugs to this type of malignancy is not known at this time. 5. **USE IN OR IMMEDIATELY PRECEDING PREGNANCY BIRTH DEFECTS IN OFFSPRING, AND MALIGNANCY IN FEMALE OFFSPRING:** The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be on the order of 1 to 4 in 1000 exposures. Although there is no evidence at the present time that oral contraceptives further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including oral contraceptives, in pregnancy. One case control study has estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortions from women who become pregnant soon after ceasing oral contraceptives. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping oral contraceptives is unknown. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period (or after 45 days from the last menstrual period if the progestogen-only oral contraceptives are used), and further use of oral contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue oral contraceptives with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend three months although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy. 6. **GALLBLADDER DISEASE:** Studies report an increased risk of surgically confirmed gallbladder disease in users of oral contraceptives and estrogens. In one study, an increased risk appeared after two years of use and doubled after four or five years of use. In one of the other studies, an increased risk was apparent between six and twelve months of use. 7. **CARBOHYDRATE AND LIPID METABOLIC EFFECTS:** A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. For this reason, pre-diabetic and diabetic patients should be carefully observed while receiving oral contraceptives. An increase in triglycerides and total phospholipids has been observed in patients receiving oral contraceptives. The clinical significance of this finding remains to be defined. 8. **ELEVATED BLOOD PRESSURE:** An increase in blood pressure has been reported in patients receiving oral contraceptives. In some women hypertension may occur within a few months of beginning oral contraceptive use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure, and in the fifth year of use is two and a half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in oral contraceptive users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given oral contraceptives. Hypertension that develops as a result of taking oral contraceptives usually returns to normal after discontinuing the drug. 9. **HEADACHE:** The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of oral contraceptives and evaluation of the cause. 10. **BLEEDING IRREGULARITIES:** Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an oral contraceptive with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of oral contraceptives. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Postuse anovulation, possibly prolonged, may also occur in women without previous irregularities. 11. **ECTOPIC PREGNANCY:** Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. 12. **BREAST FEEDING:** Oral contraceptives given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of oral contraceptives should be deferred until the infant has been weaned. **PRECAUTIONS: General:** 1. A complete medical and family history should be taken prior to the initiation of oral contraceptives. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, oral contraceptives should not be prescribed for longer than one year without another physical examination being performed. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking oral contraceptives should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. 4. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving oral contraceptive therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. Oral contraceptive users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by oral contraceptive therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping oral contraceptives, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of oral contraceptive therapy when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing oral contraceptives: a. Increased sulfobromophthalen retention b. Increased prothrombin and factors VII, VIII, IX, and X c. Decreased antithrombin 3. increased norepinephrine-induced platelet aggregability c. Increased thyroid-binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test. **INFORMATION FOR THE PATIENT: (See Patient Package Insert).** **DRUG INTERACTIONS:** Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin, griseofulvin, and tetracycline. **CARCINOGENESIS, PREGNANCY, NURSING MOTHERS: See CONTRAINDICATIONS AND WARNINGS. ADVERSE REACTIONS:** An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS): Thromboembolism. Pulmonary embolism. Coronary thrombosis. Cerebral thrombosis. Cerebral hemorrhage. Hypertension. Gallbladder disease. Liver tumors. Congenital anomalies. There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis. Neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea, usually the most common adverse reaction. Vomiting, occurs in approximately 10% or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Dysmenorrhea. Amenorrhea during and after treatment. Temporary infertility after discontinuance of treatment. Edema. Chloasma or melasma which may persist. Breast changes: tenderness, enlargement, and secretion. Change in weight (increase or decrease). Change in cervical erosion and cervical secretion. Possible diminution in lactation when given immediately postpartum. Gynecomastia. Jaundice. Migraine. Increase in size of uterine leiomyomata. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives, and the association has been neither confirmed nor refuted: Premenstrual-like syndrome. Cataracts. Changes in libido. Chorea. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiforme. Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. Hemolytic uremic syndrome. **OVERDOSAGE:** Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

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OJ-782



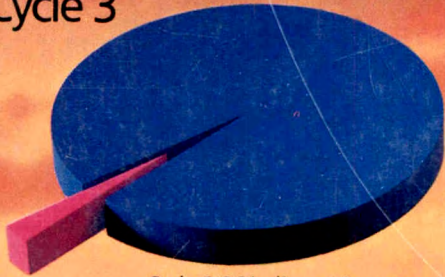
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†Serious as well as minor side effects have been reported with the use of oral contraceptives. The physician should remain alert to the earliest manifestations of any symptoms of serious disease and discontinue oral contraceptive therapy when appropriate. Please see complete Prescribing Information, a summary of which appears on the preceding page.

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- ▼ 29% lower progestin dose than 1/35<sup>1</sup>
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- ▼ Supplied in the patient-preferred Wallette™ pill dispenser.<sup>4</sup>

Serious as well as minor side effects have been reported following the use of all oral contraceptives. These include thromboembolic disease. Please see brief summary of full prescribing information on next page.

1. 1/35 formulations contain 1.0 mg norethindrone with 0.035 mg ethinyl estradiol. BTB comparisons are based on the first three cycles of use. Data available from Syntex Laboratories, Inc.
2. Wynn V, Niththyananthan R: The effect of progestins in combined oral contraceptives on serum lipids with special reference to high-density lipoproteins. *Am J Obstet Gynecol* 142:766-772, 1982.
3. Wynn V: Effect of duration of low-dose oral contraceptive administration on carbohydrate metabolism. *Am J Obstet Gynecol* 142:739-746, 1982.
4. In an independent survey the Wallette™ pill dispenser was preferred to the Ortho Dialpak by 7 out of 10 prospective OC patients. Data available from Syntex Laboratories, Inc.



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**BREVCON® 21-Day Tablets** (norethindrone 0.5 mg with ethinyl estradiol 0.035 mg)

**BREVCON® 28-Day Tablets** (21 norethindrone 0.5 mg with ethinyl estradiol 0.035 mg tablets followed by 7 inert tablets)

**NORINYL® 1 + 35 21-Day Tablets** (norethindrone 1 mg, with ethinyl estradiol 0.035 mg)

**NORINYL® 1 + 35 28-Day Tablets** (21 norethindrone 1 mg, with ethinyl estradiol 0.035 mg tablets followed by 7 inert tablets)

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**Indications:** Prevention of pregnancy. **DOSE-RELATED RISK OF THROMBOEMBOLISM:** Because studies have shown a positive association between OC use and risk of thromboembolism, it is prudent to minimize estrogen exposure. Prescribe an OC with the least amount of estrogen compatible with an acceptable pregnancy rate and patient acceptance. Start new users on OCs containing 0.05 mg or less of estrogen.

**Contraindications:** 1. Known or suspected pregnancy (see Warning #5). 2. Thrombophlebitis or thromboembolic disorders. 3. Past history of deep vein thrombophlebitis or thromboembolic disorders. 4. Undiagnosed abnormal genital bleeding. 5. OCs should not be used by women who have or have had any of the following: a cerebral vascular or coronary artery disease, including myocardial infarction, b. known or suspected carcinoma of the breast, c. known or suspected estrogen dependent neoplasia, d. benign or malignant liver tumor that developed during use of OCs or other estrogen containing products.

**WARNINGS:** Cigarette smoking increases the risk of serious cardiovascular side effects from OC use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use OCs should be strongly advised not to smoke.

The use of OCs is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, liver tumor, gall bladder disease, visual disturbances, fetal abnormalities, and hypertension. Practitioners prescribing OCs should be familiar with the following information relating to these risks.

1. **Thromboembolic Disorders and Other Vascular Problems:** An increased risk of thromboembolic and thrombotic disease associated with OC use is established. One study demonstrated an increased relative risk for fatal venous thromboembolism and several studies demonstrated it for non-fatal venous thromboembolism. They estimate that OC users are 4-11 times more likely than nonusers to develop these diseases without evident cause. One British study reported an excess death rate of 40% in OC users, most of which resulted from cardiovascular disease. Another British study showed a lower death rate in OC users than controls; an increase in cardiovascular deaths was seen but was not statistically significant. A U.S. prospective study failed to disclose increased mortality rates from cardiovascular disorders, but a subset analyzed as a retrospective, case-control study showed significant increases in venous thromboembolism. **CEREBROVASCULAR DISORDERS:** Two American studies demonstrated an increased relative risk for stroke in OC users in prospective studies. In an American study of cerebrovascular disorders in women with and without predisposing causes, relative risk of hemorrhagic stroke was estimated as 2.0 times greater and thrombotic stroke as 4-9.5 times greater in users than nonusers. A British long-term, follow-up study reported in 1976 a highly significant association between OC use and stroke. Another study had suggested an association between OC use and stroke, but the number of cases was too small to estimate the risk. Subarachnoid hemorrhage has been shown to be increased by OC use in British and American studies. Smoking alone increases incidence of these accidents; smoking and pill use appear to increase risk more than either alone. **MYOCARDIAL INFARCTION (MI):** Increased relative risk of MI associated with OC use has been reported. One British study found that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preclampsia/torax) the higher the risk of developing MI, regardless of OC use. OCs were an additional risk factor. In terms of relative risk, it has been estimated that nonsmoking OC users (smoking is considered a major predisposing condition to MI) are twice as likely to have a fatal MI as nonsmoking nonusers. OC users who are smokers have a 5-fold increased risk of fatal infarction compared to nonsmoking users, and a 10-12-fold increased risk compared to nonsmoking nonusers. The number of cigarettes smoked is important. In determining the importance of these relative risks, baseline rates for various age groups must be considered. (Estimates are based on British vital statistics which show acute MI death rates 2-3 times less than in the U.S.; so U.S. death rates could be higher.) Importance of other predisposing conditions in determining relative and absolute risks has not been quantified, other synergistic actions may exist. **RISK OF DEATH:** Using data from several national adverse reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to estrogen dose in OCs. OCs containing 0.1 mg or more of estrogen were associated with a higher risk of thromboembolism than those containing 0.05-0.08 mg but quantity of estrogen may not be the sole factor. This was supported by a U.S. study. A British study found a positive association between dose of progestogen or estrogen and certain thromboembolic conditions. Swedish authorities noted decreased reporting of thromboembolic episodes when higher estrogen preparations were no longer prescribed. Careful epidemiological studies to determine degree of thromboembolic disease risk associated with progestogen-only OCs have not been done. Thromboembolic disease has been reported in women using these products, and they should not be considered free of excess risk. **PERSISTENCE OF RISK:** Two studies have suggested an increased risk may persist for 6 years after discontinuation of OC use for cerebrovascular disease and 9 years for MI. Another study suggested persistence of risk for subarachnoid hemorrhage. **ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES:** A large British prospective study estimated mortality rate per 100,000 women per year from circulatory system diseases for OC users and nonusers according to age, smoking habits, and duration of use. The overall annual excess death rate for OC users was estimated to be 20,000,000 (ages 15-34—5,100,000; ages 35-44—33,100,000; ages 45-49—140,100,000). Risk is concentrated in long-term users and in smokers, and may persist after OC discontinuation. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all occurred in women 35 or older. An update provided the following rates: ages 15-34—1,670 for nonusers and 1,200 for smokers; ages 45 and over—1,250 for nonusers and 1,500 for smokers. Risk appeared to increase with parity, but not with duration of use. Until more women under 35 with continuous use for 5 or more years are available, it is not possible to assess relative risk for this age group. Data from a variety of sources have been analyzed to estimate risk of death associated with various methods of contraception; Estimates include combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease for OCs) plus risk attributable to pregnancy or abortion if the method fails (which varies with the effectiveness of the contraceptive method). Data are shown in Table 1 below. The study concluded that mortality associated with all contraceptive methods is below that of childbirth, except for OCs in women over 40 who smoke. (Rates given for pill only smokers for each age are for smokers as a class. For "heavy" smokers (more than 15 cigarettes a day), rates would be about double, for "light" smokers (less than 15), about half.) The lowest

mortality is with the condom or diaphragm backed up by early abortion. The study also concluded that OC users who smoke, especially over 30, have greater mortality risk than OC users who do not smoke.

**Table 1. Risk of thromboembolic and thrombotic disease associated with OCs increases with age after 30 and, for MI, is further increased by hypertension, hyperlipidemia, obesity, diabetes, or history of preclampsia/torax, and especially by smoking. The following chart gives a gross estimate of risk of death from circulatory disorders associated with OC use.**

SMOKING HABITS AND OTHER PREDISPOSING CONDITIONS—RISK ASSOCIATED WITH USE OF OCs			
Age	Below 30	30-39	40+
Heavy smokers	C	B	A
Light smokers	D	B	A
Nonusers	D	B	A
(no predisposing conditions)	D	C, D	C
Nonusers	D	C, D	C
(other predisposing conditions)	C	C, B	B, A

A—Use associated with very high risk.  
B—Use associated with high risk.  
C—Use associated with moderate risk.  
D—Use associated with low risk.

Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, discontinue OC immediately. A 4-fold increased risk of liver, biliary, and pancreatic complications has been reported in OC users. If feasible, discontinue OCs at least 4 weeks before surgery associated with increased risk of thromboembolism or prolonged immobilization. Before resuming OC after major surgery or bedrest, balance risks of post-surgery thromboembolic complications with contraceptive needs. Data suggest varicose veins substantially increase risk of superficial venous thrombosis of the leg, the risk depending on severity of the varicosities. 2. **Ocular Lesions:** Neuro-ocular lesions such as optic neuritis or retinal thrombosis have been associated with OC use. Discontinue OC if there is unexplained, sudden or gradual, partial or complete loss of vision, onset of proptosis or diplopia, papilledema, or retinal vascular lesions; and institute appropriate diagnostic and therapeutic measures. 3. **Carcinoma:** Long-term continuous administration of natural or synthetic estrogen in certain animals increases certain tumors, benign or malignant, such as breast, cervix, vagina, uterus, ovary, pituitary and liver. Certain synthetic progestogens, none currently in OCs, increase the incidence of mammary nodules, benign and malignant, in dogs. Several retrospective case-control studies reported an increased relative risk (3.1-13.9 times) associating endometrial carcinoma with prolonged use of estrogens in postmenopausal women. One publication reported the first 30 cases submitted to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the adenocarcinomas found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding when OCs were first given, polycystic ovaries), nearly all occurred in women who had used sequential OCs, which are no longer marketed. No statistical association has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs, although individual cases have been reported. Studies have shown no increased risk of breast cancer to OC or estrogen users. One study found no overall increased risk of breast cancer in OC users but a greater risk was suggested for OC users with documented benign breast disease and for long-term (2-4 years) users. Another study found a history of breast cancer among grandmothers or aunts was significantly more frequent among breast cancer patients who had used an OC continuously for one or more years than among nonusers with breast cancer. Other study indicated an increasing risk of breast cancer in OC users with long-term use, especially those who increased with duration of follow-up. One author reported that extended (over 6 years) OC use prior to first full term pregnancy was associated with a significant relative risk of breast cancer. A reduced occurrence of benign breast tumors in OC users has been well documented. One study reported malignant melanoma more frequently in OC users than controls and suggested an increased incidence of urinary tract and thyroid cancers. A prospective study of women with a history of melanoma found no increase in risk and conversion to cancer in situ in OC users compared with nonusers. This became statistically significant after 3-4 years of use. Nonuniversal of dysplasia within the first 6 months of pill use was suggested to predict progression after prolonged exposure. One study disclosed an increased risk of cancer of the cervix (largely carcinoma in situ) in OC users under 40, particularly those who had used OCs for 4 years. There have been other reports of microinvasive hyperplasia of the cervix in OC users. One study reported an association between OC use and endocervical adenocarcinoma. In summary, there is no confirmed evidence from human studies of increased risk of cancer associated with OCs. Close clinical surveillance of all OC users is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, take appropriate diagnostic measures to rule out malignancy. Monitor OC users with a strong family history of breast cancer or who have breast of the breast, fibrocystic disease, or abnormal mammograms with particular care. 4. **Liver Tumors:** Sudden severe abdominal pain or shock may be due to rupture and hemorrhage of a liver tumor. There have been reports associating benign or malignant liver tumors with short-term and long-term OC use. One study reported use of OCs with high hormonal potency and age over 30 may further increase risk of hepatocellular adenoma. Two studies relate risk with duration of use, risk being much greater after 4 or more years of use. Long-term OC users have an estimated annual incidence of hepatocellular adenoma of 2-4/100,000. Although an uncommon lesion, it should be considered in women presenting with an "acute abdomen." The tumor may cause serious or fatal hemorrhage. Patients with liver tumors have demonstrated variable clinical features which may make preoperative diagnosis difficult. Some cases presented because of upper quadrant masses, while most had signs and symptoms of acute intraperitoneal hemorrhage. Routine radiological and laboratory studies may not be helpful. Liver scans may show a focal defect. Hepatic arteriography may be useful in diagnosing primary liver neoplasms. 5. **Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring:** Use of female sex hormones—estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. Females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1/1000 exposures or less. Although there is no evidence that OCs further enhance the risk of developing this type of malignancy, such OC users should be monitored with particular care. A high percentage of women exposed to diethylstilbestrol (30-90%) have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are a precursor of vaginal malignancy. Male children so exposed may develop urogenital tract abnormalities. Although similar data are not available on other estrogens, it cannot be presumed that they would not induce similar changes. Increased risk of congenital anomalies, including heart and limb defects, has been reported following use of sex hormones, including OCs, in pregnancy. One case-control study estimated a 4.7-fold increased relative risk of limb-reduction defects in infants exposed in utero to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some exposures involved only a few days of treatment. Data suggest risk of limb-reduction defects in exposed fetuses is somewhat less than 1/1000 live births. In a large prospective study, cardiovascular defects in children born to women who received female hormones, including OCs, during early pregnancy occurred at 16.2/1000 births, compared to 7.8/1000 in children not so exposed in utero. These results are statistically significant. A Welsh study found a statistically significant excess of neural tube defects among offspring of prior OC users (within 3 months) than among controls. The incidence of two births may be increased for women who conceive shortly after discontinuing OC use. In the past, female sex hormones were used during pregnancy in an attempt to treat threatened or habitual abortion. There is evidence that estrogens are ineffective and that there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. If a woman has not adhered to the prescribed schedule, consider possible pregnancy at the first missed period (or 45 days from the last menstrual period if progestogen-only OCs are used) and discontinue OC use until pregnancy has been ruled out. For any patient who has missed two consecutive periods, rule

out pregnancy before continuing the OC. If pregnancy is confirmed, tell the patient about potential risks to the fetus and discuss advisability of continuing the pregnancy. Women who discontinue OCs to become pregnant should use an alternate form of contraception for a period of time before attempting to conceive. A 3-month period is supported by a study suggesting increased frequency of neural tube defects in women impregnated during the first 3 months after cessation of OC use. Do not use progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding as a test for pregnancy. 6. **Gall Bladder Disease:** Studies report increased risk of gall bladder disease in OC or estrogen users. In one study, an increased risk appeared after 2 years of use and doubled after 4-5 years. In another study, an increased risk was apparent between 6 and 12 months. 7. **Carbohydrate and Lipid Metabolic Effects:** Because a decrease in glucose tolerance has been observed in a significant percentage of patients on OCs, prediabetic and diabetic OC users should be carefully observed. An increase in triglycerides and total phospholipids has been observed in OC users but its clinical significance is unknown. 8. **Elevated Blood Pressure:** An increase in blood pressure has been reported with OC use. Hypertension may occur within a few months of beginning OCs. In the first year of use, incidence of hypertension may be no higher in OC users than in nonusers. Incidence in users increases with exposure and in the fifth year of use is 2-3 times that in the first year. OC use is strongly correlated with hypertension in OC users. Women with a history of elevated blood pressure (hypertension), preexisting renal disease, history of toxemia or elevated blood pressure during pregnancy, familial tendency to hypertension or its consequences, or history of excessive weight gain or fluid retention during the menstrual cycle may be more likely to develop elevated blood pressure when given OCs and should be monitored closely. Even though elevated blood pressure within the "normal" range, closely watch elevations, particularly for women with other risk factors, or for cardiovascular disease, or stroke. High blood pressure may or may not persist after OC discontinuation. 9. **Headache:** Discontinue OC and evaluate the cause of onset or exacerbation of migraine or development of a new pattern of headache which is recurrent, persistent, or severe. 10. **Bleeding Irregularities:** Breakthrough bleeding, spotting, and missed menses often make users discontinue OCs. In treatment of oligomenorrhea or secondary amenorrhea, or young women without regular cycles may tend to remain anovulatory or to become amenorrheic after OC discontinuation. Women with these preexisting problems should be advised of this and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may occur in women without previous irregularities. A higher incidence of galactorrhea and of pituitary tumors (e.g., adenomas) has been associated with amenorrhea in former users compared with nonusers. One study reported a 16-fold increased incidence of pituitary prolactin-secreting tumors among patients with postpill amenorrhea when galactorrhea was present. 11. **Fertility:** There is evidence of impairment of fertility in women discontinuing OCs in comparison with other contraceptive methods, which appears to be independent of duration of use. While impairment diminishes with time, there is an appreciable difference in results in nulliparous women for OC or other groups 30 months after discontinuation of birth control. For parous women the difference is not apparent 30 months after cessation of contraception. 12. **Ectopic Pregnancy:** Ectopic as well as intrauterine pregnancy may occur in OC failures. In progestogen-only OC failures, the ratio of ectopic to intrauterine pregnancies is higher than in nonusers, since the drugs are more effective in preventing intrauterine than ectopic pregnancies. 13. **Breast Feeding:** OCs in the postpartum period may interfere with lactation by decreasing quantity and quality of breast milk. A history of OC use prior to or during lactation has been identified in mothers receiving OCs. Effects, if any, on the breast-fed child have not been determined. If feasible, defer OC use until the infant has been weaned. **Precautions:** **GENERAL 1.** Take a complete medical and family history before starting OCs. Pretreatment and periodic physical exams should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant lab tests. As a general rule, OCs should not be prescribed for long-term use without another method of birth control. 2. Discontinue OCs if estrogen-progestogen preparations, preexisting uterine leiomyomata may enlarge. 3. Observe patients with a history of psychic depression and discontinue OCs if depression recurs to a serious degree. Patients becoming significantly depressed while taking OCs should stop the OC and use an alternate method of contraception to determine whether the symptom is drug related. 4. 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# An established standard of efficacy

in serious pelvic infection

- Clinically effective against *Bacteroides fragilis* and other anaerobes commonly found in polymicrobial infections.

- Clinically effective against many gram-positive aerobes (eg, *Staphylococcus aureus*, group B streptococci) encountered in polymicrobial infections.

Clindamycin has been associated with *Clostridium difficile* colitis as have many other antibiotics (eg, cephalosporins, penicillins, and ampicillin). See Warnings in summary of prescribing information on the adjacent page.

**Cleocin  
Phosphate**<sup>®</sup> STERILE SOLUTION  
(clindamycin phosphate injection)

900 mg q8h

Postcesarean endomyometritis  
(artist's interpretation)

**Upjohn**



A Century  
of Caring  
1886-1986



CLEOCIN PHOSPHATE® Sterile Solution  
CLEOCIN HCl® Capsules  
(clindamycin)

#### WARNING

Clindamycin therapy has been associated with severe colitis which may end fatally. Therefore, it should be reserved for serious infections where less toxic antimicrobial agents are inappropriate, as described in the Indications Section. It should not be used in patients with nonbacterial infections, such as most upper respiratory tract infections. Studies indicate a toxin(s) produced by *Clostridia* is one primary cause of antibiotic associated colitis. Cholestyramine and colestipol resins have been shown to bind the toxin *in vitro*. See WARNINGS section. The colitis is usually characterized by severe, persistent diarrhea and severe abdominal cramps and may be associated with the passage of blood and mucus. Endoscopic examination may reveal pseudomembranous colitis.

When significant diarrhea occurs, the drug should be discontinued or, if necessary, continued only with close observation of the patient. Large bowel endoscopy has been recommended.

Antiperistaltic agents such as opiates and diphenoxylate with atropine (Lomotil) may prolong and/or worsen the condition. Vancomycin has been found to be effective in the treatment of antibiotic associated pseudomembranous colitis produced by *Clostridium difficile*. The usual adult dose is 500 milligrams to 2 grams of vancomycin orally per day in three to four divided doses administered for 7 to 10 days. Cholestyramine or colestipol resins bind vancomycin *in vitro*. If both a resin and vancomycin are to be administered concurrently, it may be advisable to separate the time of administration of each drug.

Diarrhea, colitis, and pseudomembranous colitis have been observed to begin up to several weeks following cessation of therapy with clindamycin.

#### INDICATIONS

Serious infections caused by susceptible anaerobic bacteria. Patients with serious infections due to susceptible strains of streptococci, pneumococci, and staphylococci in whom its use should be reserved for penicillin-allergic patients or other patients for whom, in the judgment of the physician, a penicillin is inappropriate.

Consider the nature of the infection and the suitability of less toxic alternatives (e.g., erythro-

mycin). Bacteriologic studies should be performed to determine the causative organisms and their susceptibility to clindamycin.

#### CONTRAINDICATIONS

History of hypersensitivity to clindamycin or lincomycin.

#### WARNINGS

See WARNING box. A toxin produced by *Clostridia* is one primary cause of antibiotic associated colitis. Cholestyramine and colestipol resins have been shown to bind the toxin *in vitro*. Mild cases of colitis may respond to drug discontinuance alone. Moderate to severe cases should be managed promptly with fluid, electrolyte and protein supplementation as indicated. Vancomycin has been found to be effective in the treatment of antibiotic associated pseudomembranous colitis produced by *Clostridium difficile*. The usual adult dosage is 500 mg to 2 grams of vancomycin orally per day in 3 or 4 divided doses for 7 to 10 days. Systemic corticoids and corticoid retention enemas may help relieve the colitis. Other causes of colitis should also be considered.

A careful inquiry should be made concerning previous sensitivities to drugs and other allergens. Because antagonism has been demonstrated between clindamycin and erythromycin *in vitro*, these drugs should not be administered concurrently. *Usage in Pregnancy:* Safety has not been established. *Usage in Newborns and Infants:* Appropriate monitoring of organ system functions is desirable. *Nursing Mothers:* Clindamycin has been reported to appear in breast milk in ranges of 0.7 to 3.8 mcg/ml. *Usage in Meningitis:* Since clindamycin does not diffuse adequately into the cerebrospinal fluid, it should not be used to treat meningitis.

**SERIOUS ANAPHYLACTOID REACTIONS REQUIRE IMMEDIATE EMERGENCY TREATMENT WITH EPINEPHRINE, OXYGEN AND INTRAVENOUS CORTICOSTEROIDS SHOULD ALSO BE ADMINISTERED AS INDICATED.**

#### PRECAUTIONS

Older patients with associated severe illness may tolerate diarrhea less well. When clindamycin is indicated in these patients, they should be carefully monitored for change in bowel frequency. Prescribe with caution in individuals with a history of gastrointestinal disease, particularly colitis and also in atopic individuals. Indicated surgical procedures should be performed in conjunction with therapy. Patients with severe renal disease and/or very severe hepatic disease accompanied by severe metabolic aberrations should be dosed with caution and serum clindamycin levels monitored during high dose therapy.

During prolonged therapy, periodic liver and kidney function tests and blood counts should be

performed. Use may result in overgrowth of non-susceptible organisms, particularly yeasts. Clindamycin has neuromuscular blocking properties and may enhance other neuromuscular blocking agents. Use with caution in patients receiving such agents. Do not inject clindamycin IV undiluted as a bolus. Dilute prior to IV administration to 300 mg per 50 ml or more of diluent. Infuse over at least 10-60 minutes. (See Dosage and Administration.) CLEOCIN HCl Capsules contain FD&C Yellow No. 5 (tartrazine) which may cause allergic-type reactions (including bronchial asthma) in certain susceptible individuals, especially in patients who also have aspirin hypersensitivity.

#### ADVERSE REACTIONS

**Gastrointestinal:** Abdominal pain, nausea, vomiting and diarrhea. (See WARNING box.)

**Hypersensitivity Reactions:** Maculopapular rash and urticaria. Generalized mild to moderate morbilliform-like skin rashes are the most frequent adverse reactions. Rare instances of erythema multiforme, some resembling Stevens-Johnson syndrome, have been reported. A few cases of anaphylactoid reactions have been reported. If a hypersensitivity reaction occurs, the drug should be discontinued. The usual agents should be available for emergency treatment. **Liver:** Jaundice and abnormalities in liver function tests have been observed. **Hematopoietic:** Neutropenia, eosinophilia, agranulocytosis and thrombocytopenia have been reported; no direct etiologic relationship to concurrent clindamycin therapy has been made. **Local Reactions:** Pain, induration and sterile abscess have been reported after intramuscular injection and thrombophlebitis after intravenous infusion. Reactions can be minimized or avoided by giving deep intramuscular injections and avoiding prolonged use of indwelling intravenous catheters. **Musculoskeletal:** Rare instances of polyarthritides have been reported. **Cardiovascular:** Rare instances of cardiopulmonary arrest and hypotension have been reported following too rapid IV infusion. (See Dosage and Administration.) **Renal:** Renal dysfunction has rarely been observed. No direct relationship has been established.

#### HOW SUPPLIED

Available as sterile solution with each ml containing clindamycin phosphate equivalent to 150 mg clindamycin base. Vials of 2 ml, 4 ml, and 6 ml.

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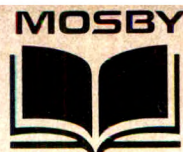


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## February

1986

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**CONTRAINDICATIONS:** Mycelex-G 500 mg Vaginal Tablets are contraindicated in women who have shown hypersensitivity to any components of the preparation.

**WARNINGS:** None.

**PRECAUTIONS:** If there is a lack of response to Mycelex-G 500 mg Vaginal Tablets, appropriate microbiological studies should be repeated to confirm the diagnosis and rule out other pathogens before instituting another course of antimycotic therapy.

**CARCINOGENESIS:** No long term studies in animals have been performed to evaluate the carcinogenic potential of Mycelex-G 500 mg Vaginal Tablets intravaginally. A long term study in rats (Wistar strains) where clotrimazole was administered orally provided no indication of carcinogenicity.

**USAGE IN PREGNANCY: PREGNANCY CATEGORY B:** The disposition of <sup>14</sup>C-clotrimazole has been studied in humans and animals. Clotrimazole is poorly absorbed following intravaginal administration to humans, whereas it is rather well absorbed after oral administration.

In clinical trials, use of vaginally applied clotrimazole in pregnant women in their second and third trimesters has not been associated with ill effects. There are, however, no adequate and well-controlled studies in pregnant women during the first trimester of pregnancy.

Studies in pregnant rats given repeated intravaginal doses up to 100 mg/kg/day have revealed no evidence of harm to the fetus due to clotrimazole.

Repeated high oral doses of clotrimazole in rats and mice ranging from 50 to 120 mg/kg resulted in embryotoxicity (possibly secondary to maternal toxicity), impairment of mating, decreased litter size and number of viable young and decreased pup survival to weaning. However, clotrimazole was not teratogenic in mice, rabbits and rats at oral doses up to 200, 160 and 100 mg/kg, respectively. Oral absorption in the rat amounts to approximately 90% of the administered dose.

Because animal reproduction studies are not always predictive of human response, this drug should be used only if clearly indicated during the first trimester of pregnancy.

**ADVERSE REACTIONS:** Of 297 patients in double-blind studies with the 500 mg vaginal tablet, 3 of 149 patients treated with active drug and 3 of 148 patients treated with placebo reported complaints during therapy that were possibly drug related. In the active drug group, vomiting occurred in one patient, vaginal soreness with coitus in another, and complaints of vaginal irritation, itching, burning and dyspareunia in the third patient. In the placebo group, clitoral irritation occurred in one patient and dysuria, described as remotely related to drug, in the other. A third patient in the placebo group developed bacterial vaginitis which the investigator classed as possibly related to drug.

Eighteen (1.6%) of the 1116 patients treated with Mycelex-G in other formulations in double-blind studies reported complaints during therapy that were possibly drug-related. Mild burning occurred in six patients while other complaints such as skin rash, itching, vulval irritation, lower abdominal cramps and bloating, slight cramping, slight urinary frequency, and burning or irritation in the sexual partner, occurred rarely.

**OVERDOSAGE:** No data available.

**DRUG ABUSE AND DEPENDENCE:** Drug abuse and dependence with Mycelex-G 500 mg Vaginal Tablets has not been reported.

**DOSE AND ADMINISTRATION:** The recommended dose is one tablet inserted intravaginally one time only, preferably at bedtime. In the event of treatment failure, that is, persistence of signs and symptoms of vaginitis after five days, other pathogens commonly responsible for vaginitis should be ruled out before instituting another course of antimycotic therapy.

**HOW SUPPLIED:** Mycelex-G 500 mg Vaginal Tablets are white, bullet shaped, uncoated tablets, coded with Miles on one side and 097 on the other, supplied as a single 500 mg tablet with plastic applicator and patient instructions.

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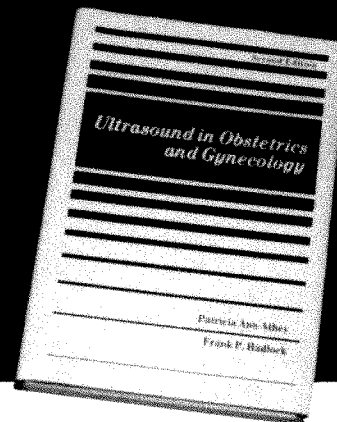
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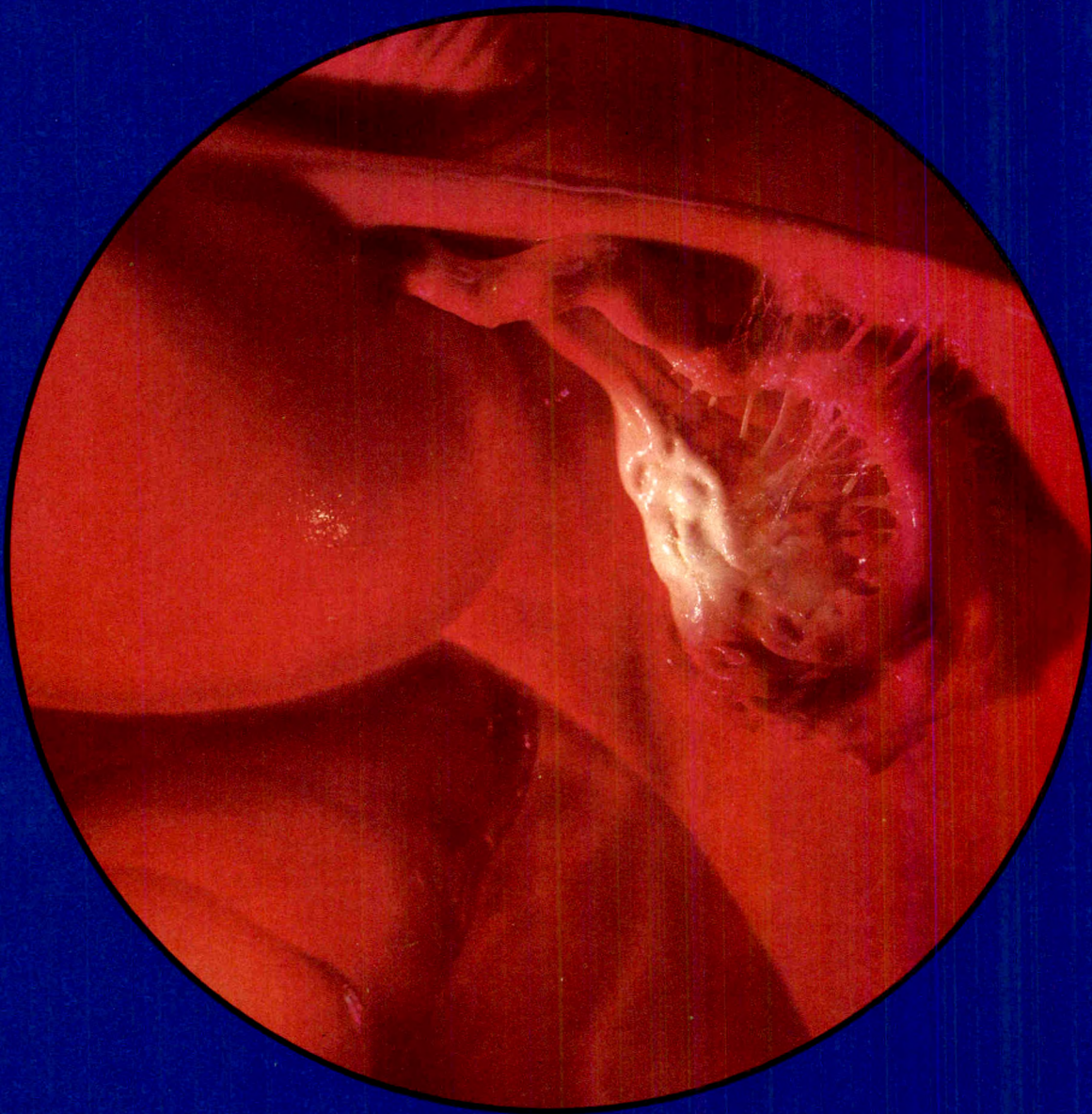
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# in pelvic infections.\*



Artist's concept of the uterus and adnexa as viewed through a diagnostic laparoscope. Pathology includes pelvic cellulitis and pelvic inflammatory disease.

\*including endometritis, pelvic cellulitis, and pelvic inflammatory disease caused by indicated organisms



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**Indications and Usage: Treatment**—Serious infections caused by susceptible strains of the designated microorganisms in the following diseases:

**LOWER RESPIRATORY TRACT INFECTIONS**, including pneumonia and lung abscess, caused by *Streptococcus pneumoniae* (formerly *Diplococcus pneumoniae*), other streptococci (excluding enterococci, e.g., *Strep. faecalis*), *Staphylococcus aureus* (penicillinase and non-penicillinase producing), *Escherichia coli*, *Klebsiella* species, *Hemophilus influenzae*, and *Bacteroides* species.

**GENITOURINARY INFECTIONS**. Urinary tract infections caused by *E. coli*, *Klebsiella* species, *Proteus mirabilis*, indole-positive *Proteus* (i.e., *P. morganii*, *P. rettgeri*, and *P. vulgaris*), and *Providencia* species. Uncomplicated gonorrhea due to *Neisseria gonorrhoeae* (penicillinase and non-penicillinase producing).

**INTRA-ABDOMINAL INFECTIONS**, including peritonitis and intra-abdominal abscess, caused by *E. coli*, *Klebsiella* species, *Bacteroides* species including the *B. fragilis* group,§ and *Clostridium* species.

**GYNECOLOGICAL INFECTIONS**, including endometritis, pelvic cellulitis, and pelvic inflammatory disease, caused by *E. coli*, *N. gonorrhoeae* (penicillinase and non-penicillinase producing), *Bacteroides* species including the *B. fragilis* group,§ *Clostridium* species, *Peptococcus* species, *Peptostreptococcus* species, and group B streptococci.

**SEPTICEMIA** caused by *Strep. pneumoniae* (formerly *D. pneumoniae*), *Staph. aureus* (penicillinase and non-penicillinase producing), *E. coli*, *Klebsiella* species, and *Bacteroides* species including the *B. fragilis* group.§

**BONE AND JOINT INFECTIONS** caused by *Staph. aureus* (penicillinase and non-penicillinase producing).

**SKIN AND SKIN STRUCTURE INFECTIONS** caused by *Staph. aureus* (penicillinase and non-penicillinase producing), *Staph. epidermidis*, streptococci (excluding enterococci, e.g., *Strep. faecalis*), *E. coli*, *P. mirabilis*, *Klebsiella* species, *Bacteroides* species including the *B. fragilis* group,§ *Clostridium* species, *Peptococcus* species, and *Peptostreptococcus* species.

Although appropriate culture and susceptibility studies should be performed, therapy may be started while awaiting these results. Cefoxitin is not active *in vitro* against most strains of *Pseudomonas aeruginosa* and enterococci (e.g., *Strep. faecalis*) and many strains of *Enterobacter cloacae*. Methicillin-resistant staphylococci are almost uniformly resistant to cefoxitin.

**Contraindications:** Previous hypersensitivity to cefoxitin and the cephalosporin group of antibiotics.

**Warnings:** BEFORE THERAPY IS INSTITUTED, CAREFUL INQUIRY SHOULD BE MADE TO DETERMINE PREVIOUS HYPERSENSITIVITY REACTIONS TO CEFOTAXIME, CEPHALOSPORINS, PENICILLINS, OR OTHER DRUGS. GIVE WITH CAUTION TO PENICILLIN-SENSITIVE PATIENTS. ANTIBIOTICS SHOULD BE ADMINISTERED WITH CAUTION TO ANY PATIENT WHO HAS DEMONSTRATED SOME FORM OF ALLERGY, PARTICULARLY TO DRUGS. IF AN ALLERGIC REACTION TO CEFOTAXIME OCCURS, DISCONTINUE THE DRUG. SERIOUS HYPERSENSITIVITY REACTIONS MAY REQUIRE EPINEPHRINE AND OTHER EMERGENCY MEASURES.

**Pseudomembranous colitis**, from mild to life-threatening in severity, has been reported with virtually all antibiotics (including cephalosporins); therefore, it is important to consider its diagnosis when diarrhea develops in association with antibiotic use. Broad-spectrum antibiotics alter normal flora of colon and may permit overgrowth of clostridia; a toxin produced by *Clostridium difficile* is a primary cause of antibiotic-associated colitis. Mild cases may respond to drug discontinuance alone; in more severe cases, management may include sigmoidoscopy, appropriate bacteriological studies, fluid, electrolyte and protein supplementation, and use of a drug such as oral vancomycin; isolation of the patient may be advisable. Other causes of colitis should also be considered.

**Precautions:** General—Total daily dose should be reduced in patients with reduced urinary output due to renal insufficiency because high and prolonged serum antibiotic concentrations can occur from usual doses. Prescribe with caution in patients with a history of gastrointestinal disease, particularly colitis. Prolonged use may result in overgrowth of nonsusceptible organisms; repeated evaluation of the patient's condition is essential. If superinfection occurs, take appropriate measures.

**Drug Interactions**—Increased nephrotoxicity has been reported following concomitant administration of cephalosporins and aminoglycoside antibiotics.

**Drug/Laboratory Test Interactions**—High concentrations (>100 mcg/mL) may interfere with measurement of serum and urine creatinine levels by the Jaffe reaction and produce false increases of modest degree in creatinine levels reported; serum samples should not be analyzed for creatinine if withdrawn within 2 hours of cefoxitin administration. High concentrations may interfere with measurement of urinary 17-hydroxy-corticosteroids by the Porter-Silber reaction and produce false increases of modest degree in levels reported. A false-positive reaction for glucose in urine has been observed with CLINITEST<sup>®</sup> reagent tablets.

**Carcinogenesis, Mutagenesis, Fertility Impairment**—No long-term animal study has been performed on carcinogenic or mutagenic potential. Rat studies at approximately three times maximum recommended human dosage revealed no effects on fertility or mating ability.

**Pregnancy Category B**—Reproduction studies in rats and mice did not reveal teratogenic or fetal toxic effects, although fetal weights were slightly decreased. In rabbits, cefoxitin was associated with a high incidence of abortion and maternal death, neither considered teratogenic. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

**Nursing Mothers**—Excreted in human milk: Exercise caution.

**Pediatric Use**—Safety and efficacy in infants from birth to three months have not yet been established. In children three months and older, higher doses have been associated with increased incidence of eosinophilia and elevated SGOT.

**Adverse Reactions:** The most common adverse reactions have been local reactions following intravenous or intramuscular injection. Other adverse reactions have been encountered infrequently. **Local Reactions**—Thrombophlebitis with intravenous administration; pain, induration, and tenderness after intramuscular injections. **Allergic Reactions**—Rash (including exfoliative dermatitis), pruritus, eosinophilia, fever, and other allergic reactions. **Gastrointestinal**—Symptoms of pseudomembranous colitis during or after treatment and, rarely, nausea and vomiting. **Blood**—Transient eosinophilia, leukopenia, neutropenia, hemolytic anemia, and thrombocytopenia; a positive direct Coombs test may develop in some individuals, especially those with azotemia. **Liver Function**—Transient elevations in SGOT, SGPT, serum LDH, and serum alkaline phosphatase. **Renal Function**—Elevations in serum creatinine and/or blood urea nitrogen levels and, rarely, acute renal failure.

**Note:** In group A beta-hemolytic streptococcal infections, therapy should be maintained for at least 10 days to guard against the risk of rheumatic fever or glomerulonephritis. In staphylococcal and other infections involving a collection of pus, surgical drainage should be carried out where indicated. Intramuscular injections should be well within the body of a relatively large muscle such as the upper outer quadrant of the buttock (i.e., gluteus maximus); aspiration is necessary to avoid inadvertent injection into a blood vessel. The total daily dosage in infants and children should not exceed 12 grams.

**How Supplied:** Sterile cefoxitin sodium in vials and infusion bottles containing 1 gram or 2 grams cefoxitin equivalent and in 10-gram bulk bottles.

§ *B. fragilis*, *B. distasonis*, *B. ovalis*, *B. theta*, *B. distans*, *B. vulgatus*.

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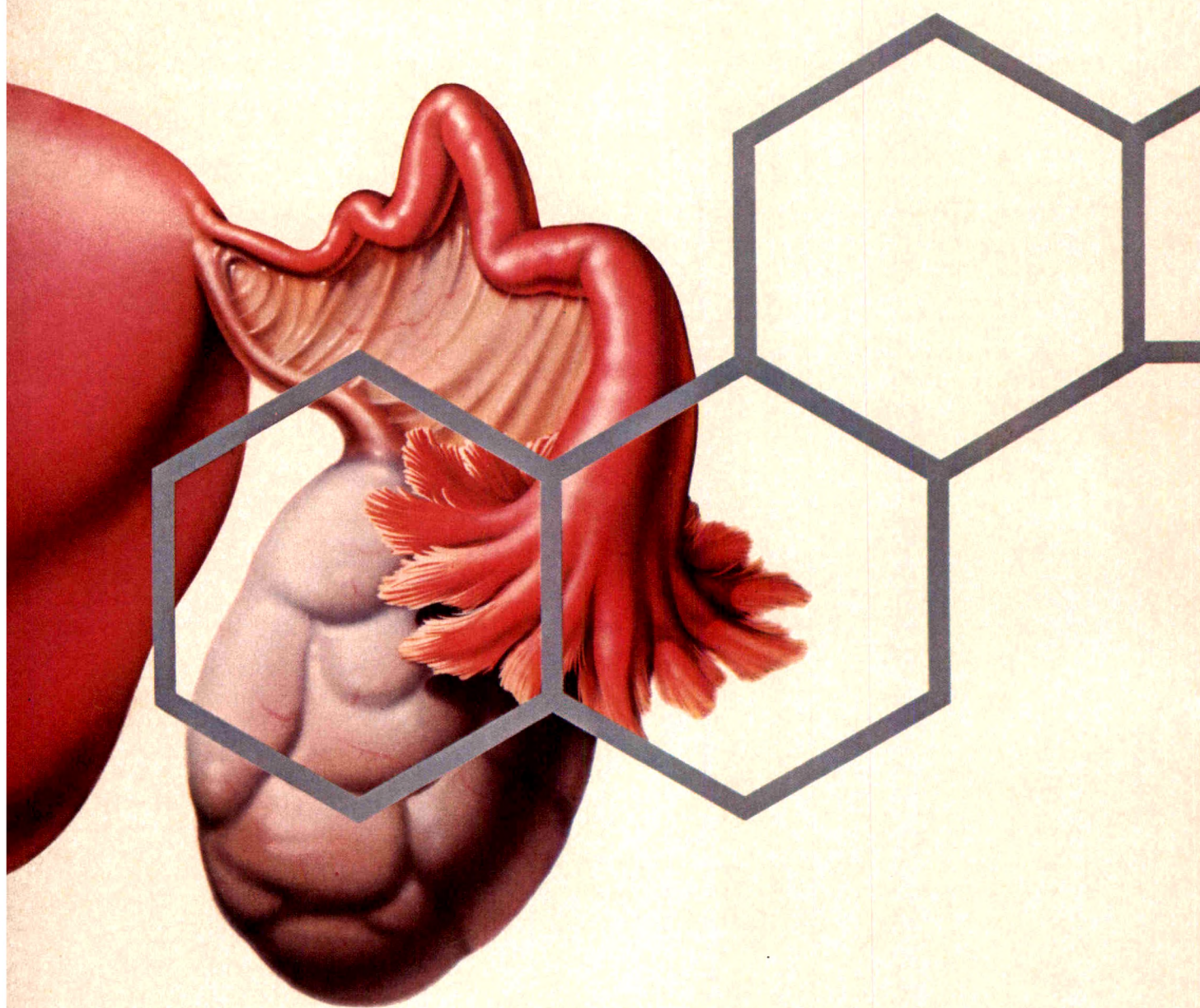
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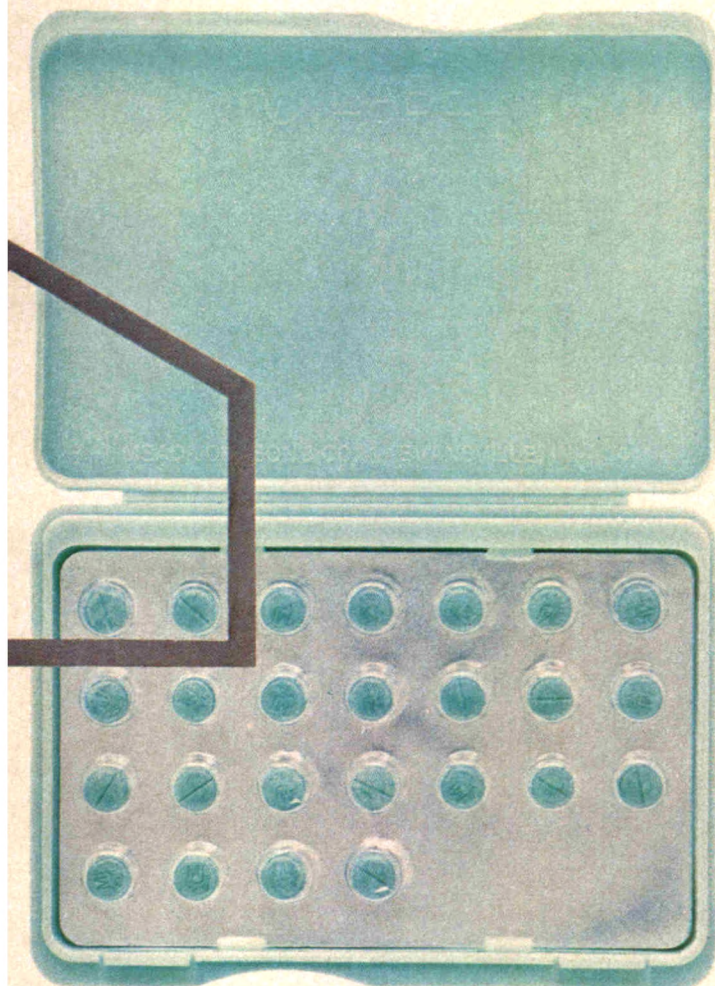
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**2. ESTROGENS SHOULD NOT BE USED DURING PREGNANCY.** The use of female sex hormones, both estrogens and progestogens, during early pregnancy may seriously damage the offspring. It has been shown that females exposed *in utero* to diethylstilbestrol, a non-steroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated as not greater than 4 per 1000 exposures. Furthermore, a high percentage of such exposed women (from 30 to 90 percent) have been found to have vaginal adenosis, epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are precursors of malignancy. Although similar data are not available with the use of other estrogens, it cannot be presumed they would not induce similar changes. Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies, including congenital heart defects and limb reduction defects. One case control study estimated a 4.7-fold increased risk of limb reduction defects in infants exposed *in utero* to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 per 1000. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well controlled studies that progestogens are effective for these uses. If ESTRACE® (estradiol) is used during pregnancy, or if the patient becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus and the advisability of pregnancy continuation.

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**Warnings:** Estrogens increase the risk of carcinoma of the endometrium (See Boxed Warning). Prescribe cautiously in women with a strong family history of breast cancer or with breast nodules, fibrocystic disease, or

abnormal mammograms. A 2- to 3-fold increase in the risk of surgically confirmed gallbladder disease has been reported in women receiving postmenopausal estrogens. There is an increased risk of thrombosis in men receiving estrogens for prostatic cancer and women for postpartum breast engorgement. If feasible, estrogen should be discontinued at least 4 weeks before surgery of the type associated with an increased risk of thromboembolism, or during periods of prolonged immobilization. Estrogens should not be used in persons with active thrombophlebitis or thromboembolic disorders or (except in treatment of malignancy) with a history of such disorders in association with estrogen use; they should be used with caution in patients with cerebral vascular or coronary artery disease and only for those in whom estrogens are clearly needed. Large doses (5 mg conjugated estrogens per day), comparable to those used to treat cancer of the prostate and breast, have been shown to increase the risk of nonfatal myocardial infarction, pulmonary embolism, and thrombophlebitis; when estrogen doses of this size are used, any of the thromboembolic and thrombotic adverse effects associated with oral contraceptives should be considered a clear risk. Hepatic adenomas should be considered in estrogen users having abdominal pain and tenderness, abdominal mass, or hypovolemic shock. Increased blood pressure occurs with use of estrogens in the menopause, and blood pressure should be monitored, especially with high doses. Diabetic patients should be carefully observed for decreased glucose tolerance. Estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases; if this occurs, the drug should be stopped and appropriate measures taken to reduce the serum calcium level.

**Precautions:** General—A complete medical and family history should be taken prior to initiation of therapy. Pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen, and pelvic organs, and should include a Papanicolaou smear. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination. Conditions influenced by fluid retention, such as epilepsy, migraine, and cardiac or renal dysfunction, require careful observation. Undesirable manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding, mastodynia, etc., may develop. Patients with a history of depression should be carefully observed. Pre-existing uterine leiomyomata may increase in size during estrogen use. Pathologists should be advised of estrogen therapy when relevant specimens are submitted. If jaundice develops, discontinue use while cause is investigated. Administer with caution in patients with impaired liver function, metabolic bone diseases associated with hypercalcemia, or renal insufficiency and in young patients with incomplete bone growth. The following endocrine and liver function tests may be affected by larger estrogen doses: increased sulfobromophthalein retention; increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability; increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by PBI, T<sub>4</sub> by column, or T<sub>4</sub> by radioimmunoassay; free T<sub>3</sub> resin uptake is decreased, reflecting the elevated TBG; free T<sub>4</sub> concentration is unaltered; impaired glucose tolerance; decreased pregnandiol excretion; reduced response to metyrapone test; reduced serum folate concentration; increased serum triglyceride and phospholipid concentration.

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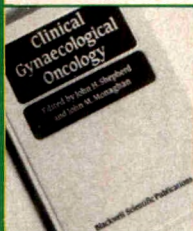
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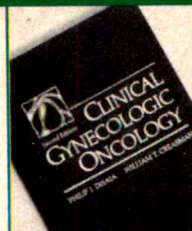


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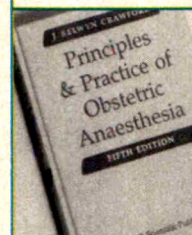


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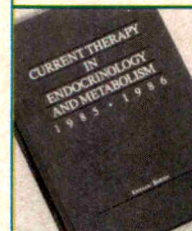


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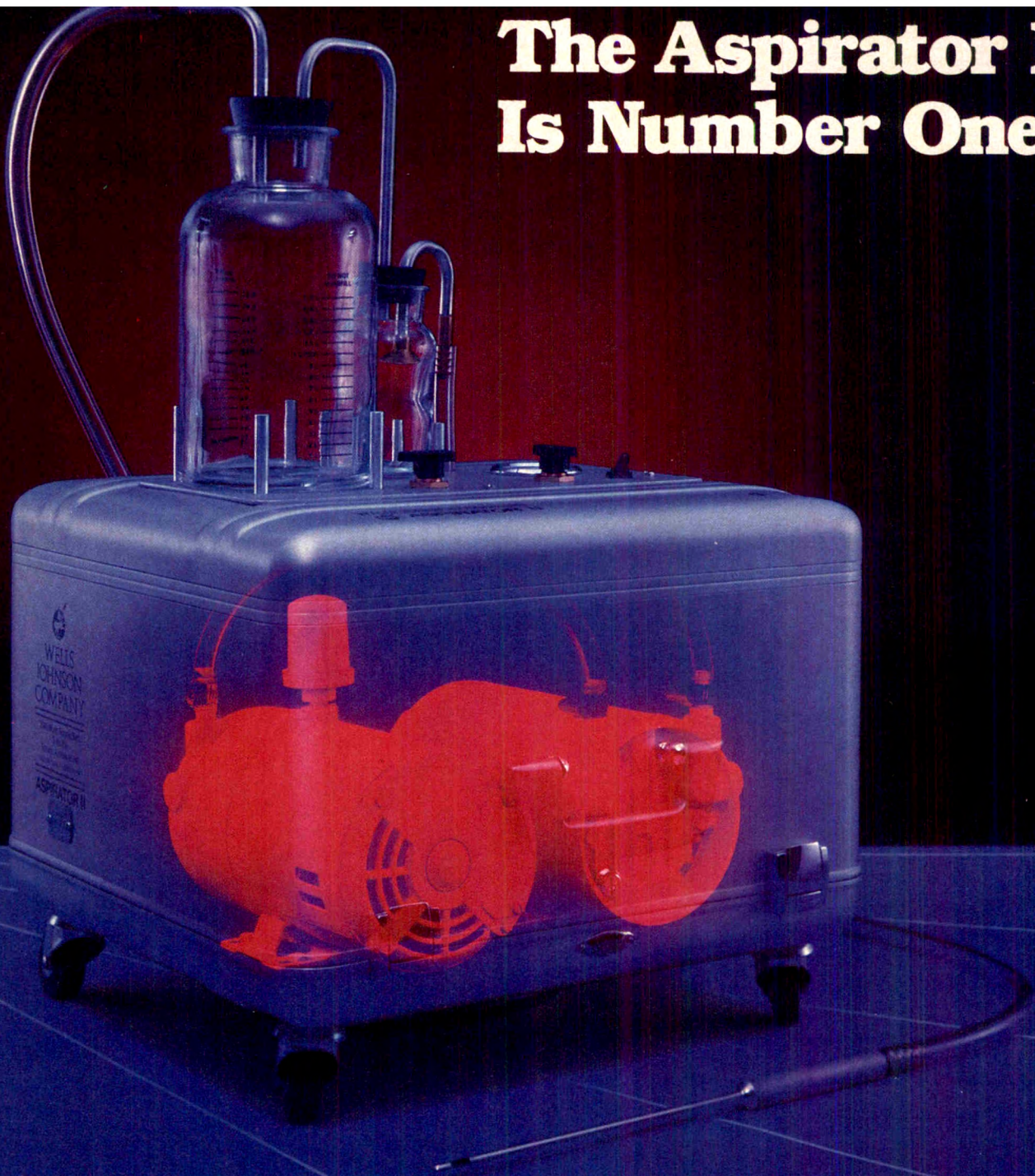
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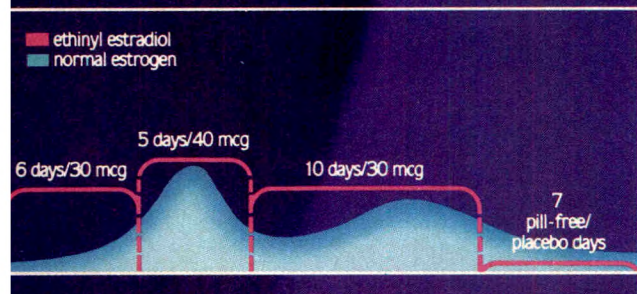




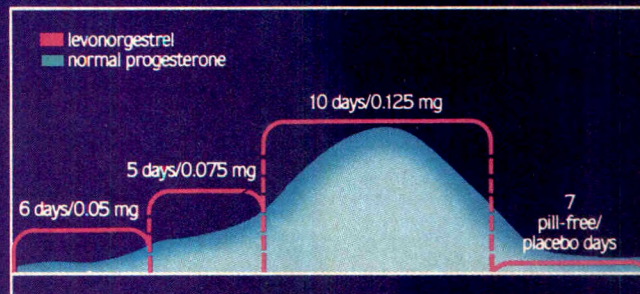
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See important information on following page.



## IN BRIEF:

**TRIPHASIL®**—6 brown tablets containing 0.050 mg levonorgestrel with 0.030 mg ethinyl estradiol; 5 white tablets containing 0.075 mg levonorgestrel with 0.040 mg ethinyl estradiol; 10 light-yellow tablets containing 0.125 mg levonorgestrel with 0.030 mg ethinyl estradiol (7 light-green tablets containing inert ingredients are included in the 28-day regimen).—Triphasic regimen.

**Indications and Usage**—TRIPHASIL® is indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OC's) as a method of contraception.

**Contraindications**—OC's should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep-vein thrombophlebitis or thromboembolic disorders. 3. Cerebral-vascular or coronary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Known or suspected pregnancy (see Warning No. 5). 8. Benign or malignant liver tumor which developed during use of OC's or other estrogen-containing products.

## Warnings

**Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.**

The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. **Thromboembolic Disorders and Other Vascular Problems**—An increased risk of thromboembolic and thrombotic disease associated with use of OC's is well established. Three principal studies in Great Britain and 3 in the U.S. have demonstrated increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OC's are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.

**CEREBROVASCULAR DISORDERS**—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

**MYOCARDIAL INFARCTION (MI)**—An increased risk of MI associated with the use of OC's has been reported, confirming a previously suspected association. These studies, conducted in the UK, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of pre-eclamptic toxemia) the higher the risk of developing MI, regardless of whether the patient was an OC user or not. OC's, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to MI) are about twice as likely to have a fatal MI as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal MI compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, amount of smoking is also an important factor. In determining importance of these relative risks, however, baseline rates for various age groups must be given serious consideration. Importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; quite likely the same synergistic action exists, but perhaps to a lesser extent.

**RISK OF DOSE**—In an analysis of data derived from several national adverse-reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to dose of estrogen in OC's. Preparations containing 100 mcg or more of estrogen were associated with higher risk of thromboembolism than those containing 50-80 mcg. Their analysis did suggest, however, that quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the U.S.

**ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES**—A large prospective study carried out in the UK estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OC's according to age, smoking habits, and duration of use. Overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), risk being concentrated in older women, in those with long duration of use and in cigarette smokers. It was not possible, however, to examine interrelationships of age, smoking, and duration of use, nor to compare effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess magnitude of relative risk for this younger group. Available data from a variety of sources have been analyzed to estimate risk of death associated with various methods of contraception. Estimates of risk of death for each method include combined risk of contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OC's) plus risk attributable to pregnancy or abortion in event of method failure. This latter risk varies with effectiveness of method. The study concluded that mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OC's in women over 40 who smoke. Lowest mortality is associated with condom or diaphragm backed up by early abortion. Risk of thromboembolic and thrombotic disease associated with OC's increases with age after about 30 and, for MI, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of pre-eclamptic toxemia, and especially cigarette smoking. Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A 4- to 6-fold increased risk of postsurgery thromboembolic complications has been reported in OC users. If feasible, OC's should be discontinued at least 4 weeks before surgery of a type associated with increased risk of thromboembolism or prolonged immobilization.

**PERSISTENCE OF RISK OF VASCULAR DISORDERS**—Findings from one study in Britain involving cerebrovascular disease and another in the U.S. concerning MI suggest an increased risk of these conditions in users of OC's persists after discontinuation of the OC's. In the British study, risk of cerebrovascular disease remained elevated in former OC users for at least 6 years after discontinuation. In the U.S. study, increased risk of MI persisted for at least 9 years in women 40 to 49 years old who had used OC's for 5 or more years. Findings in both studies require confirmation since they are inconsistent with other published information.

2. **Ocular Lesions**—There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with use of OC's. Discontinue OC's if there is unexplained, sudden or gradual, partial or complete loss of vision, onset of proptosis or diplopia, papilledema, or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. **Carcinoma**—Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in OC's, have been noted to increase incidence of mammary nodules, benign and malignant, in dogs. In humans, 3 case-control studies have reported an increased risk of endometrial carcinoma associated with prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OC's. Of cases found in women without predisposing risk factors (e.g., irregular bleeding at the time OC's were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These are no longer marketed. No evidence has been reported suggesting increased risk of endometrial cancer in users of conventional combination or progestogen-only OC's. Several studies have found no increase in breast cancer in women taking OC's or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women on OC's, found an excess risk in subgroups of OC users with documented benign breast disease. Reduced occurrence of benign breast tumors in users of OC's has been well documented. In summary, there is at present no confirmed evidence from human studies of increased risk of cancer associated with OC's. Close clinical surveillance of all women on OC's is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or with breast nodules, fibrocystic disease, or abnormal mammograms should be monitored with particular care if they elect to use OC's.

4. **Hepatic Tumors**—Benign hepatic adenomas have been found to be associated with use of OC's. One study showed that OC's with high hormonal potency were associated with higher risk than lower potency OC's. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users. Two studies relate risk with duration of use of OC's, the risk being much greater after 4 or more years' use. While hepatic adenoma is rare, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women on OC's. Relationship of these drugs to this type of malignancy is not known.

5. **Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring**—Use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have increased risk of developing in later life a form of vaginal or cervical cancer ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence now that OC's further enhance risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OC's. Furthermore, 30 to 80% of such exposed women have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with use of other estrogens, it cannot be presumed they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with use of sex hormones, including OC's, in pregnancy. One case-control study estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OC's, hormonal withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some exposures involved only a few days. Data suggest that risk of limb-reduction defects in exposed fetuses is somewhat less than 1 in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence

from well-controlled studies that progestogens are effective. There is some evidence that triploidy and possibly other types of polyplody are increased among abortuses from women who become pregnant soon after ceasing OC's. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OC's is unknown. It is recommended that, for any patient who has missed 2 consecutive periods, pregnancy should be ruled out before continuing OC's. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at time of first missed period, and further use of OC's should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and advisability of continuation of the pregnancy should be discussed. It is also recommended that women who discontinue OC's with intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this. The administration of progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. **Gallbladder Disease**—Studies report increased risk of surgically confirmed gallbladder disease in users of OC's and estrogens. In one study, increased risk appeared after 2 years' use and doubled after 4 or 5 years' use. In one of the other studies, increased risk was apparent between 6 and 12 months' use.

7. **Carbohydrate and Lipid Metabolic Effects**—Decrease in glucose tolerance has been observed in a significant percentage of patients on OC's. For this reason, prediabetic and diabetic patients should be carefully observed while on OC's. Increases in triglycerides and total phospholipids have been observed in patients on OC's. Three studies were performed with Triphasil and no significant alterations in lipid metabolism were noted except for a slight increase in triglyceride levels in 1 study. Clinical significance of these findings remains to be defined.

8. **Elevated Blood Pressure**—Increase in blood pressure has been reported in patients on OC's. In some women, hypertension may occur within a few months of beginning OC's. In the 1st year of use, prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. Prevalence in users increases, however, with longer exposure, and in the 5th year of use is 2½ to 3 times the reported prevalence in the 1st year. Age is also strongly correlated with development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure on OC's. Hypertension that develops as a result of taking OC's usually returns to normal after discontinuing the drug.

9. **Headache**—Onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of OC's and evaluation of the cause.

10. **Bleeding Irregularities**—Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing OC's. In breakthrough bleeding, as in all cases of irregular vaginal bleeding, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or change to another OC may solve the problem. Changing to an OC with a higher estrogen content, while potentially helpful in minimizing menstrual irregularity, should be done only if necessary, since this may increase risk of thromboembolic disease. Women with past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuing OC's. Women with these preexisting problems should be advised of this possibility and encouraged to use other methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. **Ectopic Pregnancy**—Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. **Breast-feeding**—OC's given in the postpartum period may interfere with lactation and decrease quantity and quality of breast milk. Furthermore, a small fraction of the hormones in OC's has been identified in the milk of mothers on OC's; effects, if any, on the breast-fed child have not been determined. If feasible, defer OC's until infant has been weaned.

**Precautions**—GENERAL—1. A complete medical and family history should be taken prior to initiation of OC's. Pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Pap smear and relevant laboratory tests. As a general rule OC's should not be prescribed for longer than 1 year without another physical examination and Pap smear.

2. Under influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size.

3. Patients with history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while on OC's should stop OC's and use an alternate method to try to determine whether the symptom is drug-related.

4. OC's may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency.

5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while on OC's. If jaundice develops, OC's should be discontinued.

6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution.

7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. Clinical significance is undetermined.

8. Serum folate levels may be depressed by OC's. Since the pregnant woman is predisposed to development of folate deficiency and incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OC's, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency.

9. The pathologist should be advised of OC therapy when relevant specimens are submitted.

10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OC's. a. Increased sulfobromophthalen retention

b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability

c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered.

d. Decreased pregnanediol excretion

e. Reduced response to metoprolol test.

**Information for the Patient**—See Patient Package Labeling.

**Drug Interactions**—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

**Carcinogenesis**—See Warnings section for information on carcinogenesis.

**Pregnancy**—Category X. See Contraindications, Warnings.

**Nursing Mothers**—See Warnings.

**Adverse Reactions**—An increased risk of these serious adverse reactions has been associated with use of OC's (see Warnings): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies. There is evidence of an association between the following conditions and use of OC's although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

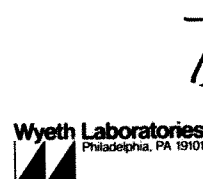
The following adverse reactions have been reported in patients on OC's and are believed to be drug-related. Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding, spotting, change in menstrual flow, dysmenorrhea, amenorrhea during and after treatment, temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement, and secretion; change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening); intolerance to contact lenses. The following adverse reactions have been reported in users of OC's, and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, hemolytic uremic syndrome.

**Acute Overdose**—Serious ill effects have not been reported following acute ingestion of large doses of OC's by young children. Overdose may cause nausea, and withdrawal bleeding may occur in females.

**Dosage and Administration**—For maximum contraceptive effectiveness, Triphasil must be taken exactly as directed and at intervals not over 24 hours. (If Triphasil is first taken later than first day of first menstrual cycle of medication or postpartum, contraceptive reliance should not be placed on it until after the first 7 consecutive days of use. Possibility of ovulation and conception prior to initiation of medication should be considered.)

Any time patient misses 1 or 2 brown, white or light-yellow tablets, she should also use another contraceptive method until she has taken a tablet daily for 7 consecutive days.

For full details on dosage and administration see prescribing information in package insert.



Levonorgestrel and ethinyl estradiol tablets—Triphasic regimen

## TRANSACTIONS OF THE FORTY-FIRST ANNUAL MEETING OF THE SOCIETY OF OBSTETRICIANS AND GYNAECOLOGISTS OF CANADA

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### Fetal cystic hygroma colli: Antenatal diagnosis, significance, and management

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Twenty-four cases of fetal cystic hygroma colli were diagnosed by ultrasound. In two patients, the diagnosis was not confirmed at autopsy. Ten of these were cases of Turner's syndrome, one was a case of Turner's mosaicism, three had other aneuploidies, four had normal chromosomes, and six had a failed chromosome culture. The diagnosis, management, and future counseling of these patients are discussed. (AM J OBSTET GYNECOL 1986;154:221-5.)

**Key words:** Fetal cystic hygroma colli, antenatal diagnosis, ultrasound, management, counseling

Recent reports have highlighted the importance of giving accurate and effective counseling to couples who have a fetal abnormality diagnosed on ultrasound.<sup>1</sup>

It had been thought that cystic hygroma colli was diagnostic of Turner's syndrome in utero and that it was the precursor of the neck webbing or redundant skin in the nuchal region found after birth.<sup>2</sup> In later studies, however, cystic hygroma was found in fetuses with other chromosome abnormalities and with normal karyotypes.<sup>3</sup> This report is a retrospective analysis of 24 pregnancies with a prenatal ultrasound diagnosis of cystic hygroma to establish the clinical significance and use of this sign as a basis for genetic counseling.

#### Patients and methods

The records of 24 women who presented from 1980 to 1984 with a prenatal ultrasound diagnosis of cystic

hygroma colli were studied. The patients either had an ultrasonographic diagnosis made by one of us (M. M.) or had been referred to the Antenatal Genetic Clinic, Toronto General Hospital, for further management, with the diagnosis made by ultrasonographers outside the clinic. In these latter cases, the ultrasonography was repeated to confirm the diagnosis. After delivery or termination of pregnancy, autopsies were carried out on all fetuses, and cytogenetic analyses were performed on amniotic fluid and/or fetal tissues.

#### Results

The mean gestation at diagnosis was 19.3 weeks (range 14 to 26 weeks). There were four intrauterine fetal deaths, the remaining 20 patients having elective termination of pregnancy on the basis of the ultrasound and/or cytogenetic findings. The autopsy results are shown in Table I.

The ultrasonic diagnosis of cystic hygroma was confirmed in 22 of the 24 cases and was accompanied by marked peripheral edema and/or effusions into the pleural, pericardial, or peritoneal cavity in 16 cases. The two fetuses in whom cystic hygroma was not confirmed at autopsy had hydrops of unknown cause.

The additional abnormal findings at autopsy are also

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Fig. 1. Fetus with large cystic hygroma.

shown. The fetus with the horseshoe kidney was female but the cytogenetic culture failed. Chromosome analysis was not available in the fetus with a single umbilical artery. The other abnormalities were all associated with aneuploid fetuses. One 45,X fetus had an adrenal neuroblastoma.

No congenital cardiac or pulmonary anomalies were found in any fetus on postmortem examination. Where hydrops fetalis was diagnosed, no cause was found at autopsy.

The results of the cytogenetic analyses are seen in Table II. In the two fetuses in whom the presence of cystic hygroma was not confirmed at autopsy, the karyotypes were 47,XX + 21 and 46,XX. In the remaining 22 fetuses, six cultures failed to grow, three of them in association with intrauterine fetal death. All six fetuses were phenotypically female. Three fetuses had normal karyotypes. In one of these cases, 500 ml of fluid had to be aspirated from the hygroma to facilitate delivery. The remaining 13 patients had abnormal karyotypes; 10 were Turner's syndrome (45,X), one Turner's mosaic (45,X/46,XX), one trisomy 18 (47,XX + 18), and one Klinefelter's syndrome (47,XXY). Five of the six nonhydropic fetuses with cystic hygroma had abnormal karyotypes; and in the remaining fetus, the culture failed to grow.

Table I. Postmortem findings in 26 cases of fetal cystic hygroma colli

<i>Autopsy findings</i>	<i>No.</i>
Cystic hygroma confirmed	22
Cystic hygroma with hydrops/ascites	16
Hydrops only	2
Renal abnormalities	3
Hydronephrosis	1
Double ureter	1
Horseshoe kidney	1
Two cord vessels	1
Absent ovary (right) and spleen	1
Adrenal neuroblastoma	1

Table II. Fetal karyotypes in 22 confirmed cases of cystic hygroma colli

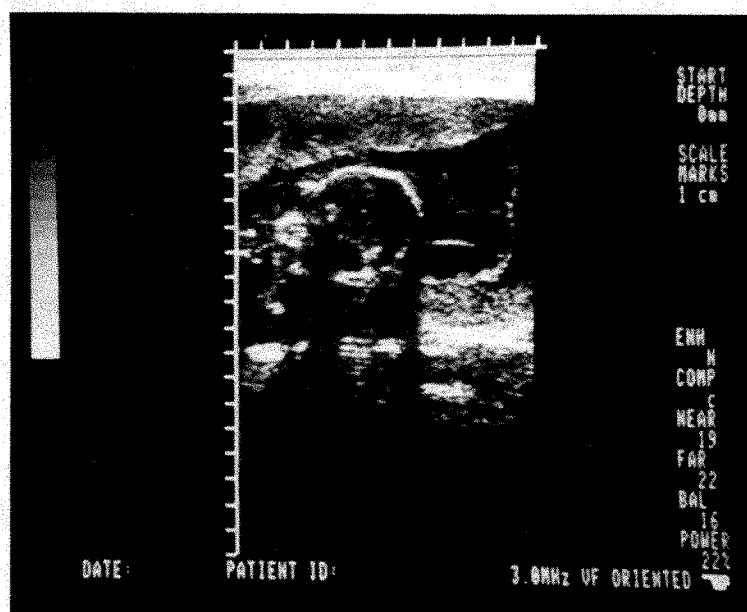
<i>Karyotype</i>	<i>No.</i>
45,X	10
(45,X)/46,XX	1
47,XX + 18	1
47,XXY	1
Failed culture	6
46,XX	2
46,XY	1

### Comment

Cystic hygroma is a congenital malformation of the lymphatic system in which dilated lymphatic channels assume cystic proportions and form a poorly defined soft tissue mass. When this occurs in the neck region (cystic hygroma colli), it covers the posterior and lateral aspects of the neck and may extend anteriorly to the anterior chest wall (Fig. 1).

It has been suggested that cystic hygroma develops as a result of failure of the jugular lymphatic sacs to drain into the internal jugular vein. This results in dilatation of the lymphatic sacs and precedes a series of events described as the jugular lymphatic obstruction sequence.<sup>4</sup> Secondary dilatation of the lymphatic channels draining the chest and limbs results in peripheral edema and hydrops. Comparison of the thoracic lymphatic duct of a normal female fetus with that of a Turner's syndrome fetus showed marked dilatation of the thoracic duct in the latter.<sup>5</sup> Further support for this theory comes from the description of a fetus with a unilateral left cystic hygroma and edema of the body with the exception of the right arm.<sup>6</sup> Singh and Carr,<sup>2</sup> in a study of eight spontaneously aborted XO fetuses, found cystic hygromas present in the three fetuses of  $\geq 77$  days' gestation (from ovulation to estimated age at death) but not in the five fetuses of shorter gestations. With the development of a link between the jugular lymphatic sac and the internal jugular vein, the sac drains, leaving redundant skin folds that give the ap-





**Fig. 2.** Ultrasound picture of cystic hygroma showing the thin-walled multiseptate, fluid-filled mass.



**Fig. 3.** Ultrasound picture of each region of hydropic fetus showing multiseptate, fluid-filled area with thick edematous skin overlying the lesion.

pearance of neck webbing seen in neonates with Turner's syndrome.

Encroachment by the dilated lymphatic ducts on the ascending aorta may produce flow changes in the fetal heart that result in the high incidence of congenital cardiac anomalies found in infants with Turner's syndrome and web neck. The most common of these is coarctation of the aorta. No cardiac anomaly was seen at autopsy in this series, four fetuses with Turner's syndrome and cystic hygroma, a further two with cystic hygroma, one with normal chromosomes, and one with failure of the chromosome culture, having had detailed examination of the heart and great vessels.

The ultrasonographic diagnosis of cystic hygroma is made by the finding of a thin-walled, multiseptate, frequently asymmetrical, fluid-filled mass attached to the lateral aspects of the fetal neck or head (Fig. 2). Generalized fetal edema may also be present. Encephalocele must be considered in the differential diagnosis.<sup>7</sup> One of the patients in this series had originally been referred with a diagnosis of occipital encephalocele, although the correct diagnosis of cystic hygroma was made on repeat ultrasound examination. Careful ultrasonographic examination of the skull and cervical spine to exclude a defect is necessary to make a precise diagnosis.

**Table III.** Karyotypes of fetal cystic hygroma colli

Series	No. in series	45,X	46,XX	46,XY	Chromosomes unknown	Trisomy 13	Trisomy 18	Trisomy 21	47,XXY
Chervenak et al. <sup>12</sup> (1983)	16	11	3	1		1			
Greenberg et al. <sup>9</sup> (1983)	1					1			
Redford et al. <sup>3</sup> (1984)	5	2		1			1	1	
Pearce et al. <sup>8</sup> (1984)	3						1	2	
Byrne et al. <sup>14</sup> (1984)	7	4	1		2				
Garden et al. (1985)	22	10 (+1 mosaic)	2	1	6		1		1
Total	54	27 (+1 mosaic)	6	3	8	2	3	3	

In our series, the main differential diagnosis was generalized hydrops. Careful review of the ultrasonic films of those fetuses with hydrops, in whom cystic hygroma was not confirmed at autopsy, showed a similar multiseptate appearance. The only differentiating sign was the presence of thick edematous skin covering the apparent cystic lesion in those patients with no cystic hygroma (Fig. 3).

The significance of an ultrasound diagnosis of cystic hygroma is not clear. Earlier reports suggested that it was diagnostic of Turner's syndrome, but recent case reports have been published linking the ultrasound finding of cystic hygroma with trisomy 18 and 21, trisomy 13, and Robert's syndrome and demonstrating it as an autosomal recessive condition.<sup>8-11</sup>

In a series of 16 fetuses, Chervenak et al.<sup>12</sup> found 11 of them to have a 45,X karyotype, one with trisomy 13, three with 36,XX, and one with 46,XY with female genitalia.

A wide range of karyotypes has been identified by us. There were 10 fetuses with Turner's syndrome, one with Turner's mosaicism (45,X/46,XX), one with trisomy 18, one with Klinefelter's syndrome, and three with normal karyotypes. In addition, the two fetuses who had an ultrasound diagnosis of cystic hygroma not confirmed at autopsy had 46,XX and 47,XX + 21 karyotypes. There was a high incidence (25%) of failed cultures. This may be subsequent to autolysis in the presence of intrauterine fetal death or the result of obtaining fluid from the cystic hygroma at the time of amniocentesis. Cystic hygroma fluid contains few cells for culture.

A review of the cytogenetic findings in series published to date is shown in Table III. Only half of the fetuses with cystic hygroma had Turner's syndrome.

In the fetuses with normal karyotypes and generalized hydrops, the diagnosis was made by routine ultrasound at 17 to 20 weeks' gestation in asymptomatic patients. The absence of presenting symptoms at this stage of gestation contrasts with findings in nonimmune hydrops fetalis in later pregnancy, where as many as 80% are complicated by hydramnios, raised blood pressure, or maternal anemia.<sup>13</sup>

Byrne et al.,<sup>14</sup> in a review of seven spontaneously aborted fetuses with cystic hygroma, found one with a small lesion but without hydrops. This fetus was the only one with a normal karyotype in their series. They suggested that the absence of hydrops in a female fetus with cystic hygroma and other anomalies associated with the 45,X karyotype might be a valid and reliable indicator of a normal chromosome complement. Six fetuses in our series had no hydrops. In this group, three fetuses had a 45,X karyotype, one was 47,XX + 18, and one 47,XXY. The remaining fetus had a failed chromosome culture but was female and had horseshoe kidneys, a combination which, in the presence of cystic hygroma, is suggestive of Turner's syndrome. Thus at least five of the six nonhydropic fetuses were aneuploid, showing that the absence of hydrops is of little diagnostic value.

The prognosis for fetuses with cystic hygroma, particularly in the presence of hydrops, is poor. In our series, five of the fetuses died before delivery, and one was noted to have a marked bradycardia prior to termination. In the series of Chervenak et al.,<sup>12</sup> nine of 15 fetuses died either in utero or in the early neonatal period. Two of the remaining six, who were electively aborted, were noted to have bradycardia. The seven cases reported by Byrne et al.<sup>14</sup> all aborted spontaneously at gestational ages of 16 to 26 weeks. Reviews of nonimmunologic hydrops where the diagnosis was usually made in the third trimester of pregnancy report a mortality rate as high as 98%.<sup>13</sup>

It can no longer be assumed that a fetus with cystic hygroma has Turner's syndrome. Chromosome analysis is required, as knowledge of the karyotype is essential for parental genetic counseling. Tissue cultures from multiple fetal sites need to be established in order to increase the likelihood of obtaining a successful karyotype.

If a normal karyotype is obtained in a hydropic fetus with an apparent cystic hygroma on ultrasound, this may be a manifestation of nonimmune hydrops. Appropriate genetic, immunologic, and viral studies are then required.

One hydropic fetus in this series had an adrenal neu-

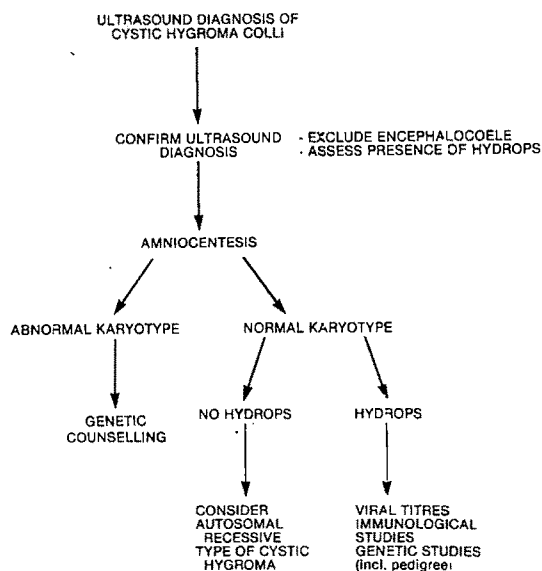


Fig. 4. Management of cystic hygroma.

roblastoma, which has been reported as a cause of generalized fetal hydrops,<sup>16</sup> possibly secondary to high catecholamine output. The karyotype was 45,X, and a cystic hygroma was present, which might also explain the generalized hydrops.

The finding of cystic hygroma colli on ultrasound during the first half of pregnancy requires careful assessment if the parents are to receive appropriate counseling. A scheme of management is shown in Fig. 4. A team approach is essential to allow accurate diagnosis by an experienced ultrasonographer and effective counseling by a clinical geneticist. This allows the parents to receive supportive and informative counseling, both regarding the affected pregnancy and in the future.

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# Amniotic fluid phosphatidylglycerol and phosphatidylcholine phosphorus as predictors of fetal lung maturity

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The contents of phosphatidylglycerol and phosphatidylcholine phosphorus in amniotic fluid (10,000 × g pellets) were studied as predictors of fetal lung maturity. The presence of phosphatidylglycerol predicted the absence of neonatal respiratory distress syndrome with 99% probability. When phosphatidylglycerol was absent, phosphatidylcholine phosphorus was a reliable predictor if measured 3 to 7 days before delivery. The probability that respiratory distress syndrome would not occur was 94% when phosphatidylcholine phosphorus was >6. When measurement was performed within 2 days of delivery, the probability that respiratory distress syndrome would not occur fell to 69%. As measured in amniotic fluid, phosphatidylglycerol and phosphatidylcholine phosphorus are reliable antenatal predictors of fetal pulmonary maturity and, therefore, are useful in the management of a number of obstetric conditions. (AM J OBSTET GYNECOL 1986;154:226-30.)

**Key words:** Amniotic fluid, fetal lung maturity, phosphatidylglycerol, phosphatidylcholine phosphorus, respiratory distress syndrome

In a variety of obstetric settings delivery of a fetus before term is desirable. However, the benefit sought by preterm delivery must be measured against the risk of neonatal respiratory distress syndrome (RDS) and its related morbidity and mortality.

Several methods of amniotic fluid analysis such as the lecithin/sphingomyelin ratio, the shake test, and, more recently, phosphatidylglycerol determinations are currently available for the assessment of fetal lung maturity.<sup>1-9</sup> Some investigators<sup>2-5</sup> have reported that a combination of tests, for instance, the lecithin/sphingomyelin ratio and phosphatidylglycerol determination, improve the predictive accuracy of any single test and, therefore, advocate such a combination of testing. In our center we have been performing phospholipid analyses on isolated surfactant fractions of amniotic fluid (10,000 × g pellets) since 1978, and, as previously reported,<sup>10</sup> we found that the phosphatidylglycerol determination on these fractions was superior to both the lecithin/sphingomyelin ratio and the shake test in predicting fetal pulmonary maturity as well as immaturity. We have accepted the presence of phosphatidylglycerol in these fractions as being indicative of fetal pulmonary maturity but have also found many instances in which

the absence of phosphatidylglycerol was not predictive of the development of RDS.

We subsequently demonstrated that the measurement of the phosphatidylcholine content of the same isolated surfactant fractions of amniotic fluid provides an index for the rate of surfactant accumulation.<sup>11</sup> With this measurement expressed as the phosphatidylcholine phosphorus value (phosphatidylcholine phosphorus content of the surfactant present in 10 ml of amniotic fluid), it was found that this value progressively increased before the appearance of phosphatidylglycerol. The hypothesis that this measurement may be of value in assessing the state of fetal pulmonary maturity in the absence of phosphatidylglycerol was therefore addressed.

## Material and methods

A review of all phospholipid profiles analyzed between 1978 and 1983 was made. Clinical variables such as intrauterine growth retardation, premature rupture of the membranes, diabetes mellitus, and the use of steroids were not excluding factors. Phospholipid profiles were measured on isolated surfactant fractions as previously described.<sup>10, 11</sup> Briefly, this involves centrifugation of a carefully measured aliquot (preferably 5 to 10 ml) of each amniotic fluid sample for 5 minutes at 140 × g to remove cellular debris followed by a 20-minute spin at 10,000 × g to pellet the surfactant particles. Lipids were then extracted from the surfactant pellets and separated by two-dimensional thin-layer chromatography. The individual phospholipids, after visualization with the use of a modified molybdenum

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spray reagent, were scraped from the plate and quantitated by measurement of their phosphorus content. When volumes other than 10.0 ml were used, the phosphorus content of phosphatidylcholine was appropriately corrected to give the phosphatidylcholine phosphorus value. Phosphatidylglycerol is routinely calculated as a percentage of total lipid phosphorus but is reported in this communication as either present or absent. By this method the limit of detection for phosphatidylglycerol is 0.2 to 0.5  $\mu\text{g}$ .

All amniocenteses were done to assess fetal lung maturity based on clinical indicators. None was done solely to collect data for the study. The decision to perform the amniocentesis was made by the individual attending obstetrician. All patient management decisions were also made by the attending obstetrician. In the earlier years of study, the shake test and the lecithin/sphingomyelin ratio were the standard accepted tests and were the basis for obstetric management decisions. After initial work on phosphatidylglycerol by Bent et al.,<sup>10</sup> phosphatidylglycerol became the standard test of fetal lung maturity at this institution. At no time was a phosphatidylcholine phosphorus value used to direct obstetric management of the patients in the study group. The study group then comprised patients who had fetal pulmonary testing performed antenatally for any indication regardless of that indication or other variables. The only exclusions were in patients with multiple gestations or patients whose infants died so soon after birth that RDS could not possibly develop. Clinical findings, indication for amniocentesis, gestational age, and neonatal outcome were recorded. All data were collected from the Grace Maternity Hospital obstetric population, which accounts for 5000 to 5500 deliveries per year. This hospital is the sole maternity unit for metropolitan Halifax and is the tertiary care obstetric center for the province of Nova Scotia and elsewhere in the Atlantic region. A total of 1678 amniotic fluid samples from 1359 patients were analyzed. Blood and/or meconium in the sample did not affect the assay.

To reevaluate phosphatidylglycerol as a predictive test only transabdominal samples of amniotic fluid taken within 2 days prior to delivery were considered. To assess phosphatidylcholine phosphorus, the same criteria were applied. However, only samples where phosphatidylglycerol was absent were considered. Because of the steady rise in phosphatidylcholine phosphorus that occurs prior to the appearance of phosphatidylglycerol, the time frame in this group was extended to include samples collected 3 to 7 days before delivery as well as those within 2 days of delivery.

RDS was diagnosed by the following criteria: grunting, tachypnea, sternal and intercostal retractions, and nasal flaring of  $\geq 6$  hours' duration and requiring treatment with oxygen. Severity was classified by degree of

**Table I.** RDS in relation to phosphatidylglycerol determination (<2 days before delivery)

Phosphatidylglycerol	No RDS	RDS
Present	592	10
Absent	28	36

$$\text{Positive predictive value} = \frac{a}{a + b} = 98.3\%.$$

$$\text{Negative predictive value} = \frac{d}{c + d} = 56.3\%.$$

$$\text{Sensitivity} = \frac{a}{a + c} = 95.5\%.$$

$$\text{Specificity} = \frac{d}{b + d} = 78.3\%.$$

$$\text{False positive rate} = \frac{b}{b + d} = 21.7\%.$$

$$\text{False negative rate} = \frac{c}{a + c} = 4.5\%.$$

$$\text{Accuracy} = \frac{a + d}{a + b + c + d} = 94.3\%.$$

$$\text{Prevalence} = \frac{a + c}{a + b + c + d} = 93.1\%.$$

$$\text{Pretest probability} = \text{prevalence} = 93.1\%.$$

$$\text{Likelihood ratio} = \frac{\text{sensitivity}}{\text{false positive rate}} = 4.4.$$

$$\text{Posttest probability (from nomogram)} = 99\%.$$

respiratory assistance required in treatment. Severe RDS required assisted mechanical ventilation, moderate RDS required  $>35\%$  oxygen and/or continuous positive airway pressure, and mild RDS required only an increase in oxygen concentration up to 35%. Diagnosis of RDS and its severity was made by the attending neonatologist who was not aware of the results of the phospholipid profile.

Analysis of the data was made with the use of standard diagnostic test parameters, that is, positive predictive value, negative predictive value, sensitivity, specificity, and accuracy. To further assess the predictive ability of the phosphatidylglycerol and phosphatidylcholine phosphorus measurements, likelihood ratios, and probability of RDS occurring or not occurring were determined.

The key to the analysis is the likelihood ratio, which expresses the odds that a given diagnostic test result (presence of phosphatidylglycerol or level of phosphatidylcholine phosphorus) would be expected in a patient with the target outcome (no RDS). This is calculated in a two-by-two table as the true positive rate (sensitivity), divided by the false positive rate (one mi-

**Table II.** RDS in relation to phosphatidylcholine phosphorus (<2 days before delivery)

Phosphatidylcholine phosphorus	No RDS	RDS
≥6	18	10
<6	10	26

Positive predictive value = 64%. Negative predictive value = 72%. Sensitivity = 64.3%. Specificity = 72%. False positive rate = 28%. False negative rate = 36%. Accuracy = 69%. Prevalence = 43.8%. Pretest probability = 43.8%. Likelihood ratio = 2.3. Posttest probability = 69%.

**Table III.** Significant RDS\* in relation to phosphatidylcholine phosphorus (<2 days before delivery)

Phosphatidylcholine phosphorus	No significant RDS	Significant RDS
≥6	25	3
<6	16	20

Positive predictive value = 89%. Negative predictive value = 56%. Sensitivity = 61%. Specificity = 87%. False positive rate = 13%. False negative rate = 39%. Accuracy = 70%. Prevalence = 64.1%. Pretest probability = 64.1%. Likelihood ratio = 4.7. Posttest probability = 89%.

\*Significant RDS = moderate or severe RDS.

nus specificity).<sup>12, 13</sup> It can also be calculated for various levels of diagnostic test result.<sup>13</sup>

To provide a more meaningful expression for clinicians, the likelihood ratio can also be used to determine the probability of the target outcome (no RDS) occurring for a given test result (that is, presence of phosphatidylglycerol or level of phosphatidylcholine phosphorus). To do this, one must have some estimate of the probability of the target outcome occurring prior to obtaining of the test result (pretest probability). In clinical obstetric practice, this might be based on experience of outcomes at similar gestations. For our present calculations, the prevalence of the target outcome in the group under study was used. This pretest probability of not developing RDS and the appropriate likelihood ratio were plotted on a nomogram<sup>14</sup> to determine the posttest probability, that is, the probability of a patient not developing RDS with a given diagnostic test result.

## Results

A total of 666 profiles were obtained from amniotic fluid within 2 days before delivery. Phosphatidylglycerol was found to be present in 602. Ten cases of RDS were found in the presence of phosphatidylglycerol.

**Table IV.** RDS in relation to phosphatidylcholine phosphorus (3 to 7 days before delivery) (pretest probability 74.5%)

Phosphatidylcholine phosphorus	No RDS	RDS	Likelihood ratio	Posttest probability of no RDS (%)
<2	2	8	0.09	19
2-6	10	4	0.85	69
>6	29	2	4.9	94

**Table V.** Significant RDS in relation to phosphatidylcholine phosphorus (3 to 7 days before delivery) (pretest probability 81.8%)

Phosphatidylcholine phosphorus	No significant RDS	Significant RDS	Likelihood ratio	Posttest probability of no RDS (%)
<2	3	7	0.10	30
2-6	11	3	0.81	79
>6	31	0	Infinity	100

Only three cases of significant (moderate to severe) RDS were found and these infants were severely asphyxiated at birth. In 64 cases in which phosphatidylglycerol was absent, there were 36 cases of RDS. The pretest probability of not developing RDS was 93.1% and the likelihood ratio was calculated to be 4.4. The posttest probability of not developing RDS when phosphatidylglycerol was present was therefore 99% (Table I).

The phosphatidylcholine phosphorus values were evaluated in the 64 cases in which the phospholipid profile revealed phosphatidylglycerol to be absent within 2 days preceding delivery. When phosphatidylcholine phosphorus was ≥6 (28 cases), 11% developed moderate or severe RDS, 25% developed mild RDS, and 64% did not develop RDS. When phosphatidylcholine phosphorus was <6 only 28% did not develop RDS while 17% developed mild RDS and 55% developed moderate or severe RDS. The pretest probability of not developing RDS when phosphatidylglycerol was absent within 2 days of delivery was 43.1%. The likelihood ratio was 2.3. From the nomogram, the posttest probability of not developing RDS when the phosphatidylcholine phosphorus was ≥6 was 69.0% (Table II).

Because of the benign nature of mild RDS, the data were similarly assessed with only moderate and severe RDS considered to be significant disease. The pretest probability of not developing significant RDS was 64.1%. The likelihood ratio was 4.7 and therefore the posttest probability of not developing significant RDS when phosphatidylcholine phosphorus was ≥6 was 89% (Table III).

There were 55 cases in which phosphatidylglycerol was absent 3 to 7 days before delivery. These were



analyzed for phosphatidylcholine phosphorus and the data for phosphatidylcholine phosphorus values of <2, 2 to 6, and >6 are presented in Table IV. The calculated likelihood ratios were found to increase as phosphatidylcholine phosphorus levels increased. Consequently, the probability of the infant not developing RDS rose from only 19% when phosphatidylcholine phosphorus was <2 to 94% when phosphatidylcholine phosphorus was >6 between 3 and 7 days before delivery.

The data pertaining to significant (moderate or severe) RDS are presented in Table V. Since there were no instances of significant RDS in any of the 31 cases where phosphatidylcholine phosphorus was >6 when measured 3 to 7 days before delivery, the likelihood ratio was calculated as infinity and the posttest probability of not developing moderate or severe RDS approached 100%.

Of the 64 cases with absent phosphatidylglycerol, 10 were in insulin-dependent diabetic women. Four had phosphatidylcholine phosphorus measured within 2 days of delivery. There was one case of moderate RDS, the phosphatidylcholine phosphorus being 1.98, in a Class C diabetic patient (White's classification). The other three all had phosphatidylcholine phosphorus >6 and none developed RDS. Six patients with diabetes had phosphatidylcholine phosphorus measured 3 to 7 days before delivery. There was one case of moderate RDS occurring in a Class B diabetic patient with a phosphatidylcholine phosphorus value of 1.38 6 days before delivery. The remaining five all had phosphatidylcholine phosphorus >6 and none developed RDS.

However, the aim was not the investigation of the status of diabetes in relation to pulmonary maturity in the fetus. The numbers are too small to make any significant observations or conclusions but the group was looked at separately simply as an interesting observation. Predictability of outcome from the test result did not change when the patients were diabetic.

Only 21 of 64 patients in the study group received steroids; no trends were noted and no conclusions could be made with these small numbers.

As expected, the majority of patients who developed significant RDS were  $\leq 34$  weeks' gestation. Ten of 24 patients >34 weeks' gestation developed significant RDS when phosphatidylglycerol was absent. This reaffirmed the need for pulmonary maturity testing.

Mode of delivery was assessed as well, but no difference could be demonstrated clinically or statistically among patients who had labor followed by spontaneous vaginal delivery, labor followed by cesarean section, or cesarean section without labor.

#### Comment

Antenatal determination of fetal pulmonary maturity is an important adjunct to optimum obstetric care.

Good pregnancy dating is aided by first- and second-trimester ultrasonography. Obviously, if the indication for delivery is such that the risk of significant RDS is less than the risk of intrauterine death, testing for fetal pulmonary maturity is not indicated. However, there are many situations where either gestation is not known or the intrauterine environment is considered to be sufficiently hostile that the best interest of the fetus would be served by delivery before term, provided fetal pulmonary maturity is assured. It is in these circumstances one seeks a predictor of the risk of RDS in the newborn infant.

Phosphatidylglycerol is the second most abundant phospholipid in mature surfactant, and, while most investigators<sup>2,7,9</sup> agree that its presence in amniotic fluid is indicative of lung maturity, many<sup>2-5</sup> advocate the use of this measurement in combination with some other test of lung maturity, most notably the lecithin/sphingomyelin ratio. Since we have found that the lecithin/sphingomyelin ratio provided no additional information to our method of phosphatidylglycerol determination,<sup>10</sup> the lecithin/sphingomyelin ratio has been discontinued at this institution since 1980. The updated data presented herein support the conclusion that the presence of phosphatidylglycerol in the isolated surfactant fraction accurately predicts an absence of RDS. It is likely that no test will ever be absolutely predictive, in that RDS may develop in mature fetuses for a number of reasons, in particular, asphyxia neonatorum and acidosis. It is noteworthy that all three cases of significant RDS from the group with phosphatidylglycerol present occurred in infants that were severely asphyxiated at birth.

In predicting the occurrence of RDS in the neonate by a lack of phosphatidylglycerol in amniotic fluid, the test appears to be less reliable. This has been demonstrated by other investigators<sup>3,5-7</sup> and, although we have found our phosphatidylglycerol method to be more reliable than the lecithin/sphingomyelin ratio in predicting lung immaturity,<sup>10</sup> both our previous and updated data indicate instances in which phosphatidylglycerol is absent and the infant does not develop RDS. As mentioned in a previous report,<sup>11</sup> it is important to note that phosphatidylglycerol is present in the surfactant fraction of amniotic fluid and is often present in very low quantities, particularly just after its initial appearance. We have often found that in order to detect these low levels a 10.0 ml aliquot of amniotic fluid must be used. Using smaller volumes of amniotic fluid or using any steps in the methodology that would decrease these already low levels of phosphatidylglycerol, that is, centrifugation beyond  $140 \times g$  to clear the sample of cells,<sup>15</sup> acetone precipitation (Oulton M., unpublished observation), or quantitation by densitometry,<sup>16</sup> would diminish the likelihood of detecting these low levels. These factors pose less of a problem in detecting

phosphatidylglycerol when it is present in much larger quantities. They do, however, provide an explanation as to why some investigators<sup>2, 3, 7, 8</sup> often fail to detect phosphatidylglycerol until other tests, such as the lecithin/sphingomyelin ratio, are mature. The high false negative rate of the phosphatidylglycerol test reported by other centers<sup>3, 5-7</sup> (which may be as high as 70% to 80%) can be attributed to these effects. It is felt that these problems can be minimized by determining phosphatidylglycerol on the isolated surfactant fractions as described in this and previous reports.<sup>10, 11</sup>

As the appearance of phosphatidylglycerol is somewhat abrupt,<sup>11</sup> it is not possible to predict with 100% accuracy exactly when it will appear. Also, there may be some delay in its appearance in amniotic fluid after its production and release to alveoli. When phosphatidylglycerol is not present in amniotic fluid, it would then be useful to have some other index of the lung maturation process. We have previously shown that the measurement of the phosphatidylcholine phosphorus value of the isolated 10,000 × g pellet provides an index of surfactant accumulation and that the initial rise in this value precedes the appearance of phosphatidylglycerol.<sup>11</sup> Therefore, we assessed the usefulness of this value in determining fetal lung maturity in instances where phosphatidylglycerol was not detectable.

If the need for delivery is considered urgent and mild RDS is not a major concern, then a phosphatidylcholine phosphorus value of  $\geq 6$  would be a strong predictor that the fetal outcome will be satisfactory insofar as RDS is concerned. There is an 89% probability that significant RDS will not occur in our setting. If delivery is not so urgent, the phosphatidylcholine phosphorus value may be even more helpful. In our study there was a 94% probability of the neonate not developing RDS if phosphatidylcholine phosphorus was  $> 6.3$  to 7 days before delivery. If mild RDS is not a concern, then the probability of avoiding significant RDS approaches 100% when the phosphatidylcholine phosphorus is  $> 6$ . It is reasonable to assume that if a full 7 days were to elapse from amniocentesis to delivery, the results would only improve. The test would then be of twofold benefit: It would allow a delivery date to be set and it would avoid the need for a repeat amniocentesis with its associated risk.

No antenatal test is 100% predictive of the presence or absence of neonatal RDS. Peripartum factors will at times change the outcome from that which is predicted. The presence of phosphatidylglycerol in amniotic fluid is an accurate predictor of fetal pulmonary maturity. To ensure the detection of the very low levels of phosphatidylglycerol that are often present, we recommend its analysis on the isolated surfactant fraction.

When phosphatidylglycerol is absent, phosphatidylcholine phosphorus is of clinical value as a predictor of fetal pulmonary maturity.

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# An in vitro fertilization and embryo transfer pilot study: Treatment-dependent and treatment-independent pregnancies

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A pilot program of in vitro fertilization and embryo transfer at McMaster University is described. Fourteen couples with a clinical diagnosis of infertility due to apparent tubal dysfunction, with evidence of ovulation, and with normal semen analysis underwent induction of superovulation with human menopausal gonadotropin. At laparoscopy, 82 follicles were aspirated and 19 oocytes were recovered. Eleven oocytes demonstrated cleavage and all inseminated oocytes were transferred 49 to 65½ hours after insemination. In vitro fertilization therapy resulted in two pregnancies, one leading to a spontaneous abortion and the other to the birth of a healthy female infant. At 10 to 12 months after in vitro fertilization therapy in the remaining 12 patients, there were three additional pregnancies (treatment-independent), one ectopic and two intrauterine. One patient was delivered of a healthy female infant. In vitro fertilization therapy should be evaluated by a randomized controlled clinical trial. (AM J OBSTET GYNECOL 1986;154:231-5.)

**Key words:** In vitro fertilization, embryo transfer, infertility, tubal dysfunction

Since the report of the birth of the first child conceived extracorporeally in 1978, there has been a marked increase in the overall success rate of the procedure.<sup>1</sup> While the first reports described a success rate of less than 2% per laparoscopy, recent reports indicate that greater success can be anticipated.<sup>2-4</sup> Despite the development of this new technique, with all the major resource implications, there have been few attempts to critically assess the comparative efficacy of this therapy in patients with nonsevere forms of infertility, that is, with patent fallopian tubes.<sup>5</sup> This report describes a pilot study in couples selected for in vitro fertilization and embryo transfer on the basis of a clinical diagnosis of an apparent tubal cause of infertility and indicates that treatment-independent pregnancy is an important outcome. In couples with nonsevere causes of infertility the efficacy of in vitro fertilization and embryo transfer should be evaluated in a randomized controlled trial.

## Material and methods

**Clinical characteristics.** Fourteen couples were selected for in vitro fertilization and embryo transfer on the basis of an apparent diagnosis of tubal infertility with normal ovulation and semen characteristics. The patients were selected from the practices of two gynecologists specializing in infertility therapy. The av-

erage age of the women was  $32.2 \pm 0.7$  years. The mean duration of infertility was  $6.6 \pm 0.8$  years with a range from 3 to 12 years. As a group, there had been 11 ectopic pregnancies and 19 major operative procedures for either tubal surgical procedures or conservative procedures for ectopic pregnancy. Two of the 14 women had previously carried pregnancies to term prior to developing secondary infertility (Table I).

**Ovulation induction.** Ovulation was induced according to the Norfolk program of increasing doses of human menopausal gonadotropin from day 3 of the menstrual cycle.<sup>3</sup> Daily ultrasonograms were obtained from day 7 of the cycle until 10,000 IU of human chorionic gonadotropin (hCG) was administered intramuscularly approximately 30 hours after the last injection of human menopausal gonadotropin (hMG). A decision to terminate hMG administration was made when the serum estradiol level reached 300 pg/ml (1101 pmol/L). Laparoscopy and oocyte recovery were undertaken 35 hours after the hCG injection. In two instances, the time from hCG administration to laparoscopy was shortened to approximately 8 to 10 hours because of impending ovulation. Aspiration of the ovarian follicles was undertaken with the use of the Monash Teflon-lined needle with a foot pedal control of wall suction at 100 mm Hg.<sup>6</sup>

**Laboratory aspects of in vitro fertilization.** With recovery of the oocyte, a semen sample was obtained from the husband 4 hours later. The specimen was prepared by use of a "swim-up" technique and approximately 100,000 sperm were added to each 3 ml of culture.<sup>7</sup> The aspiration medium, insemination medium, and growth medium were all tested in a mouse embryo

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**Table I.** Clinical characteristics

Patient No.	Age (yr)	G-T-P-A-L-E*	Infertility (yr)	Surgical procedures	Status of tubes
Treatment-independent pregnancies					
1	35	1-0-0-1-0-0	3 yr	Giant myomectomy	Peritubal adhesions, open
2	35	4-1-0-0-1-2	4 yr	Salpingectomy, left	Peritubal adhesions, open
3	30	1-0-0-0-0-1	5 yr	Partial salpingectomy, right Salpingectomy Salpingostomy	Peritubal adhesions, open
Treatment-related pregnancies					
1	31	2-0-0-0-0-2	6 yr	Salpingectomy	Severe clubbing
2	32	0-0-0-0-0-0	6 yr	Partial salpingectomy Conservative surgical procedure Severe endometriosis	Peritubal adhesions, open left
No pregnancies					
1	35	2-0-0-0-0-2	3 yr	Salpingectomy (partial salpingectomy, tubal reanastomosis)	Blocked
2	34	1-0-0-0-0-1	7 yr	Salpingectomy	Blocked
3	32	5-1-0-1-1-3	4 yr	Salpingostomy Salpingectomy	Blocked
4	29	0-0-0-0-0-0	7 yr	Partial salpingectomy, salpingectomy Bilateral reanastomosis, left reanastomosis	Open, adhesions
5	32	0-0-0-0-0-0	9 yr	Bilateral salpingostomy	Blocked
6	28	1-0-0-1-0-0	12 yr	Salpingolysis	Blocked
7	32	0-0-0-0-0-0	5 yr	Salpingolysis Conservative procedure for endometriosis, salpingectomy	Adhesions, blocked
8	32	0-0-0-0-0-0	8 yr	Salpingolysis Salpingolysis	Blocked
9	36	0-0-0-0-0-0	12 yr	Salpingostomy Salpingolysis	Adhesions, patent

\*G = Gravidity; T = total children delivered; P = premature deliveries; A = abortions; L = living children; E = ectopic pregnancies.

**Table II.** In vitro fertilization and embryo transfer in 14 patients

Couples enrolled	14
Laparoscopies	13
Successful aspirations	10 (76.9%)
Total No. of follicles aspirated	82
Total No. of oocytes	19 (23%)
Immature oocytes	3 (15.8%)
Mature oocytes	16 (84.2%)
Oocytes demonstrating cleavage	11 (57.8%)
No. of embryos	11
Embryos degenerating in vitro	1 (9%)
Embryos transferred	10 (91%)
Four embryos	1
Three embryos	1
Two embryos	3
One embryo	5
No. of pregnancies	2
Pregnancy rate per laparoscopy	15%
Pregnancy rate per fertilized oocyte	28%

system prior to use to ensure quality control. The aspirating fluid was Dulbecco's phosphate buffered saline plus 10 IU of heparin per milliliter. Insemination medium was Ham's F 10 plus 7.5% human cord serum. The growth medium was Ham's F 10 plus 15% human cord serum. All media were sterilized by filtration through a Nalgene filter unit (0.2  $\mu$ m). The incubation took place in a 5% carbon dioxide in air incubator at

37° C. Once the embryo was seen to have undergone cleavage, the embryo(s) was taken up in a Monash embryo transfer catheter in 30  $\mu$ l of medium and transferred to the patient in the operating room. Embryo return was accomplished by placing the patient in a lithotomy or knee chest position in order to make the uterine fundus dependent. The catheter containing the embryos was directed into the uterine cavity to a distance that had been predetermined by ultrasonography. The embryos contained in approximately 10  $\mu$ l of growth medium were released approximately 0.5 cm from the fundus of the uterus. The patient remained in either a supine or a prone position for several hours prior to returning home on a schedule of reduced activity.<sup>8</sup>

**Luteal phase.** In order to compensate for large numbers of granulosa cells lost during the repeated irrigation of all moderate-sized follicles, 25 mg of progesterone was given by intramuscular injection on a daily basis until pregnancy ensued. At that point, hydroxyprogesterone caproate (Delalutin), 250 mg once a week, was administered.

## Results

Of the 14 couples enrolled in the program, 13 had in vitro fertilization and embryo transfer (Table II).

**Table III.** Outcomes of therapy and 12-month follow-up

Patient No.	No. of follicles (day 0)*	Estradiol (day 0)* (pmol/L)	No. of follicles aspirated	Oocytes		Embryos	Outcome of IVF	Follow-up, 10-12 mo
				I	M			
Treatment-independent pregnancies								
1	4	2455	5	0	2	2	Biochemical pregnancy	Pregnant at 4 mo after IVF; cesarean section, female infant
2	4	1587	7	0	2	2	No pregnancy	Ectopic pregnancy at 1 mo after IVF
3	2	916	5	0	1	1	No pregnancy	Intrauterine pregnancy at 11 mo after IVF
Treatment-dependent pregnancies								
4	4	1711	6	0	2	2	Pregnant; female infant	For repeat IVF
5	5	1720	6	0	4	4	Early abortion, severe ITP	For repeat IVF
No pregnancy								
6	4	201	3	0	1	1	No pregnancy	For repeat IVF
7	2	1128	3	0	1	1	No pregnancy	Declined repeat IVF
8	4	3000	9	0	3	3	No pregnancy	Declined repeat IVF
9	3	1493	4	0	0	0	No oocytes	For repeat IVF
10	4	2473	10	0	0	0	No oocytes	For repeat IVF
11	6	1827	0	0	0	0	Ovulated spontaneously	For repeat IVF
12	5	1498	7	2	0	2	No pregnancy	For repeat IVF
13	9	1717	9	0	0	0	No oocytes	For repeat IVF
14	4	1689	7	1	0	1	No pregnancy	For repeat IVF

I = Immature; M = mature; IVF = in vitro fertilization; ITP = idiopathic thrombocytopenic purpura.

\*Day 0 = day of human chorionic gonadotropin administration.

One woman ovulated prior to laparoscopy. Of the remaining couples, 10 had successful oocyte recovery, and the total number of oocytes recovered was 19 from 82 aspirated follicles. All oocytes were fertilized and 11 of the 19 demonstrated cleavage. One embryo subsequently degenerated and the 10 remaining embryos were transferred to the women.

The serum estradiol levels were not predictive of successful recovery or cleavage. The estradiol levels at the time of the hCG injection in patients with a successful recovery, with evidence of fertilization, and with evidence of failure of cleavage were  $2690 \pm 283$ ,  $2744 \pm 219$ , and  $2567 \pm 924$ , respectively ( $p > 0.05$ ).

While ultrasound, which was carried out on the day before the hCG injection, indicated the presence of a total of 33 follicles  $>14$  mm in diameter, in fact, a total of 82 follicles were aspirated. The 19 oocytes were obtained in one to eight follicle washes. The median number of tubes for a successful aspiration was three. Five follicle washes accounted for  $>90\%$  of the oocytes recovered. In six patients with both ovaries accessible, oocytes were obtained in all, whereas in those patients with only one ovary accessible, eggs were obtained in four of seven cases.

In two cases, immature oocytes were obtained and these underwent a delayed insemination at  $10\frac{1}{2}$  and  $37\frac{1}{2}$  hours. Embryo transfer was similarly delayed by several hours. Embryo transfer was undertaken between 49 and 69 hours after insemination.

Two pregnancies occurred during the program (Table III). In one, shortly after fertilization, the pa-

tient developed a generalized hemorrhagic diathesis due to a severe recurrence of idiopathic thrombocytopenic purpura. The generalized bleeding disorder also involved the genital tract and terminated a progressive rise in serum levels of the  $\beta$ -subunit of hCG. The second patient conceived and had an uneventful pregnancy with the exception of labor complicated by bleeding, which was subsequently shown to be due to a subchorial hemorrhage. The infant was female with a birth weight of 2865 gm.

Currently, at a point 10 to 12 months from the start of the in vitro fertilization treatment month, three additional patients have conceived (Table III). One patient has had a third ectopic pregnancy; two others have had intrauterine pregnancies and one of these has been delivered of a healthy female infant by cesarean section. The other has a healthy intrauterine pregnancy with no evidence of difficulty (Table III).

### Comment

This pilot study of in vitro fertilization and embryo transfer was successful in obtaining one live infant in a total of 14 couples, and one spontaneous abortion occurred simultaneously with generalized hemorrhagic diathesis due to idiopathic thrombocytopenic purpura. This represents a 15% pregnancy rate per laparoscopy and a 28% pregnancy rate per cleaved embryo. The recovery of oocytes per follicle aspirated was somewhat low, 23%, yet the cleavage rate per oocyte was reasonable at 57% (Table II).<sup>9</sup>

Despite these successes, the additional three preg-

nancies that occurred after the in vitro fertilization cycle deserve comment. One patient had had removal of a giant myoma causing bilateral isthmic obstruction. The patient had 3 years of infertility after this operative procedure. While both tubes were patent at preliminary laparoscopy, there were mild peritubal adhesions involving both adnexa. This patient subsequently conceived and underwent a cesarean section with delivery of a live-born infant. At this time, the peritubal adhesions were confirmed and were seen to involve primarily the left tube.

The second patient to conceive did so in the second month after the in vitro fertilization therapy. This patient had had two previous ectopic pregnancies involving both fallopian tubes. A conservative operative procedure had been done on the remaining left tube. A recurrent ectopic pregnancy occurred after the in vitro fertilization cycle. This ectopic pregnancy occurred at the point at which the conservative salpingostomy had been performed. At the patient's request, a further conservative procedure was done.

The third patient conceived spontaneously 9 months after the in vitro fertilization cycle. This patient had had one previous salpingectomy for an ectopic pregnancy and had had a salpingostomy carried out. This tube was significantly involved with pelvic adhesions, although it was patent at the time of the preliminary laparoscopy. At the present time, an intrauterine pregnancy has been confirmed on ultrasound with a fetal heartbeat present.

These findings are consistent with several previous reports in the literature indicating that spontaneous "cure" of infertility can occur despite long durations of childlessness.<sup>11, 12</sup> In a retrospective study of couples followed up 36 to 48 months after registration, more than 60% of pregnancies occurred without or long after the cessation of treatment. With the advent of in vitro fertilization and embryo transfer, it is quite possible that a similar trend may exist among these couples. The indications of in vitro fertilization and embryo transfer are expanding beyond complete tubal obstruction. Several reports have recommended the use of in vitro fertilization and embryo transfer for idiopathic infertility, endometriosis, and oligospermia.<sup>3, 14</sup> In Canada, where the costs of health care for in vitro fertilization and embryo transfer therapy are not borne 100% by government insurance, couples are determining their own indications for such therapy by their economic status. Concern has already been expressed regarding the costs and cost-effectiveness of this therapy.<sup>15-17</sup> The broadening of the indications to include less severe causes of infertility will require additional economic study.

The findings of this pilot study and the 10 to 12

months of follow-up of the couples involved indicate several important factors in the management of couples with in vitro fertilization and embryo transfer. First, spontaneous "cure" of infertility is still possible despite long standing infertility and a relatively serious factor affecting fecundity.

Additionally, because such spontaneous cures of infertility due to an apparent tubal cause can occur, it may be prudent not to either coagulate both fallopian tubes or remove them prior to in vitro fertilization therapy if the tubes are patent. This practice has been advocated to eliminate the possibility of ectopic pregnancy.

Finally, as in any normal therapy, the true value of the therapy in couples with nonsevere forms of infertility can only be determined by a randomized clinical trial of in vitro fertilization and embryo transfer therapy compared to a period without therapy. Such a trial is currently underway at the Chedoke McMaster Hospitals Fertility Clinic.

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## Birth asphyxia, trauma, and mortality in twins: Has cesarean section improved outcome?

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The outcome of two populations of twins delivered at the same hospital, numbering 554 in 1963 to 1972 and 614 in 1978 to 1984, was reviewed to determine the factors contributing to depression at birth, trauma, and mortality in each period. The cesarean section rate had increased from 3% in the early period to 51% in the later period, with 92% of the later cases in which the first twin presented abnormally being delivered by cesarean section. Among infants of >28 weeks' gestation the incidence of severe depression at birth was not reduced with the increased cesarean rate, remaining at 2% in both populations; none developed encephalopathy or died as a result of birth asphyxia or trauma. Neonatal mortality was markedly reduced in the second period, primarily because of a reduction in deaths resulting from respiratory distress syndrome. It is not possible to show that the marked increase in the rate of cesarean delivery has improved the condition of twin infants at birth. (*AM J OBSTET GYNECOL* 1986;154:235-9.)

**Key words:** Twins, birth asphyxia, trauma, mortality, cesarean section

Developments in obstetric and neonatal care have resulted in a marked improvement in perinatal mortality rates. Twin pregnancies continue to pose a significant problem, with a perinatal mortality sixfold to tenfold higher than that for singletons.<sup>1-4</sup> Prematurity, with its associated respiratory distress syndrome, is the leading cause of twin death.<sup>1,5,6</sup>

Birth asphyxia is considered to be a major risk for twins. In recent years indications for cesarean section have been generally liberalized in an attempt to minimize trauma and asphyxia for the baby. Twins, with their frequent malpresentations, have experienced a particularly high rate of cesarean section.

The present study was undertaken to determine to what extent a liberal policy for cesarean section in twin pregnancies, especially those with malpresentations, has affected the incidence of birth asphyxia and trauma. Populations of twins delivered in the same hos-

**Table I.** Population studied

Gestational age (wk)	No. of infants	
	1963-1972	1978-1984*
<29	22	30
29-30	20	24
31-32	22	52
33-34	60	76
35-36	68	134
≥37	312	264
Unknown	50	34
Total	554	614

\*Includes 152 births after antenatal referral.

pital before and after the cesarean section rate increased were studied. In addition, mortality rates and causes of death were reviewed during both periods to determine which conditions had responded to recent developments in perinatal care and which remain as challenges for the future.

### Population and methods

The Royal Victoria Hospital is a tertiary care teaching institution with obstetric and neonatal intensive care facilities. The case histories studied were unselected twin pregnancies consecutively delivered at this hospital

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**Table II.** Cesarean section rate by presentation (1978 to 1984)\*

Presentation		n	Total cesarean sections (%)	Cesarean sections for malpresentation (%)
Twin I	Twin II			
Vertex	Vertex	130	20	2
Vertex	Malpresentation	92	57†	36
Malpresentation	Vertex	33	91	76
Malpresentation	Malpresentation	50	92	80
Total		305	51	33

\*Two sets of twins were not included, since presentation was not indicated.

†Includes nine sets of whom the first twin was delivered vaginally and the second twin by cesarean section; in eight sets the indication was malpresentation.

**Table III.** Incidence of depression at birth in two time periods (excluding 15 stillborn twins)

Gestational age (wk)	Twin order	1963-1972		1978-1984		p
		No. of live births	Incidence (%)	No. of live births	Incidence (%)	
≥37	First	154	3.9	131	3.1	NS
	Second	155	11.0	130	8.5	NS
29-36	First	84	15.5	139	23.0	NS
	Second	83	33.7	141	27.0	NS

during two periods. There were 554 twins delivered to 277 mothers in 1963 to 1972, and 614 twins delivered to 307 mothers in 1978 to 1984. The latter group consisted of 462 infants delivered to mothers whose original obstetric care was by physicians attached to this hospital and 152 infants delivered to mothers referred in premature labor by physicians attached to other hospitals. These antenatal referral patients, other than being premature, had similar management and outcome as those initially at this hospital. They are therefore included in analyses, although comparisons are made separately for term and preterm infants to avoid distortions resulting from the higher incidence of prematurity in the population treated in 1978 to 1984 (Table I).

During the 5-year interval between the two study periods cesarean section, which previously was seldom performed for twin pregnancies, became the method of choice for delivery in about one-half of all twin pregnancies. Other significant changes in obstetric management at that time included increasing use of ultrasound during pregnancy (allowing an earlier diagnosis of twins) and intrapartum fetal monitoring, along with steroid prophylaxis for respiratory distress syndrome. Improvements also occurred in neonatal intensive care, particularly with respect to mechanical ventilation and parenteral nutrition.

All obstetric and neonatal hospital records were coded and entered into the computer soon after the patient's discharge. A standardized diagnostic classification of neonatal disorders has been employed throughout the 21-year period, with all neonatal charts reviewed by one of the authors (R. U.), who was also

directly responsible for the care of most of the sick infants.

Clinical "depression" at birth was defined as infant respiratory depression sufficient to require positive-pressure ventilation to establish regular sustained respirations after birth; the term "severe depression" was used when an infant required more than 3 minutes of ventilation.<sup>7</sup> Analyses of depression were restricted to babies delivered after 28 weeks' gestation, since almost all babies born earlier required ventilation. Traumatic outcomes included fractures and peripheral nerve injuries (palsies). The term "malpresentation" was defined as all presentations other than vertex. Statistical analyses were by  $\chi^2$ .

Perinatal deaths were analyzed at the time of death, and a summary was prepared which included attribution of primary cause of death. The autopsy rate exceeded 90%. All births of ≥500 gm were included. Neonatal mortality in this paper included infants dying at any age before discharge home, whether death occurred in this hospital, after transfer to a pediatric center for surgery, etc.

## Results

Delivery was by cesarean section for 3.2% of twins delivered in 1963 to 1972 and 50.8% in 1978 to 1984. The higher cesarean rate in the later period was primarily for malpresentations. There was a 92% cesarean rate when the first twin was a malpresentation and a 57% rate for second twins who were malpresentations compared to a 20% cesarean rate where there was a vertex presentation for both twins (Table II).

Depression at birth varied with maturity and twin

**Table IV.** Incidence of depression at birth by method of delivery

	Vaginal delivery		Caesarean section		<i>p</i>	Incidence of depression in all births (%)
	No. of live births	Incidence of depression (%)	No. of live births	Incidence of depression (%)		
≥37 weeks' gestation						
Vertex presentation						
First twin	190	2.1	39	7.7	NS	3.1
Second twin	128	5.5	24	4.2	NS	5.3
Malpresentation						
First twin	21	14.3	26	0	NS	6.4
Second twin	77	22.1	47	8.5	NS	16.9
29-36 weeks' gestation						
Vertex presentation						
First twin	142	14.1	26	38.5	.005	17.9
Second twin	88	18.2	28	32.1	NS	21.6
Malpresentation						
First twin	11	18.2	46	34.8	NS	31.6
Second twin	60	41.7	50	38.0	NS	40.0

**Table V.** Anesthesia and depression at birth in twins delivered by cesarean section in the absence of fetal hazard\*

Gestational age (wk)	Epidural anesthesia		General anesthesia		<i>p</i> †
	No. of live births	Incidence of depression (%)	No. of live births	Incidence of depression (%)	
≥37	82	2.4	25	12.0	NS
33-36	55	18.2	24	41.7	0.05
29-32	17	52.9	14	71.4	NS

\*Indication for cesarean was malpresentation or previous cesarean section.

†Combined significance with use of Mantel-Haenszel  $\chi^2$  summation = <0.005.

order (Table III). The incidence of depression in the early period when delivery was almost always vaginal rose from 4% in term first twins to 34% in preterm second twins. The increased cesarean section rate in the later period was not accompanied by a reduction in incidence of depression at birth either among term or preterm infants, or among first or second twins.

The 286 infants delivered by cesarean section after 28 weeks' gestation in the later period were then compared with infants of similar maturity delivered vaginally in both periods combined (454 from the early period and 263 from the later) to determine the effect of cesarean birth on the incidence of depression at birth (Table IV). Subgroups of the same gestational age, presentation, and twin order were compared. The 16 infants delivered by cesarean section in the early period could not be included, since their type of presentation was not stated. Cesarean section delivery was not associated with a reduction in the rate of depression in any group.

In several groups of infants, especially preterm ones, the rate of depression was higher after cesarean birth (Table IV). The contribution of general anesthesia to this depression is evident (Table V) when infants de-

**Table VI.** Mortality rates (per 1000)

Mortality	1963-1972	1978-1984
Total births of ≥500 gm*		
Neonatal	64.3	47.2 (37.3)†
Fetal	18.1	17.9 (13.0)
Perinatal	81.2	65.1 (49.8)
≥29 weeks, excluding lethal anomalies‡		
Neonatal	27.4	10.4 (2.3)
Fetal	13.3	13.9 (11.2)
Perinatal	40.3	24.3 (13.5)

\*Number of births in 1963-1972 = 554 and in 1978-1984 = 614.

†Parentheses indicate mortality rates for Royal Victoria Hospital population of 462 infants (445 at ≥29 weeks), excluding antenatal referrals.

‡Cases in which menstrual history was unknown and the gestational age was estimated from birth weight. Number of births in 1963-1972 = 528 and in 1978-1984 = 576.

livered by cesarean section for indications without fetal hazard are analyzed according to type of anesthesia employed. Even when preterm infants were delivered by cesarean section under epidural anesthesia, however, the rate of depression (26% of 72 infants, Table V) was not lower than when preterm infants with mal-



**Table VII.** Perinatal deaths by cause in two time periods (500g or more, including deaths until discharge from hospital)

	<29 weeks*		≥29 weeks*	
	1963-1972	1978-1984†	1963-1972	1978-1984†
No. of births	24	33‡	530	581‡
Cause of death				
Respiratory distress syndrome	12	5	10	2
Twin-to-twin transfusion	0	2	1	5
Anomaly	0	0	2	5
Birth asphyxia	1	5	0	0
Fetal malnutrition	0	1	4	1
Necrotizing enterocolitis	0	0	1	3
Unexplained antepartum stillbirth	3	0	1	0
Miscellaneous	4	4	6	3
Total deaths	20	21	25	19

\*Unknowns were assigned a gestational age on the basis of weight.

†Births after antenatal referral are included in 1978-1984 data.

‡One twin died in utero before 29 weeks whereas the other twin was live born after 29 weeks, which resulted in the uneven numbers.

presentations were delivered vaginally (15% of 71 infants, Table IV).

Difficulty in delivery sufficient to produce severe depression was studied in both periods. The overall incidence of severe depression among infants of gestational age greater than 28 weeks, excluding referrals, was similar: 10 cases or 2.1% in the early period and 9 cases or 2.0% in the later period. An attempt was made to assess the contribution of delivery-related asphyxia and trauma to severe depression by studying those cases in which other maternal or fetal causative factors were not present. Thirty severely depressed infants were delivered in both periods, including 11 cases among referral patients. Of these, only 13 could not be attributed to factors existing before the delivery: fetal malnutrition, severe preeclampsia, twin-to-twin transfusion, or congenital anomalies. Analysis of these 13 showed that difficult vaginal breech delivery was a factor in only four cases, general anesthesia may have played a role in five others, and four were due to other or unknown causes.

Encephalopathy as a result of birth asphyxia or trauma was not seen in any of the twins. Fractures and peripheral nerve injuries were found in one infant during the early period and in four during the later period. In three of the cases a malpresentation occurred; one case was delivered vaginally and two by cesarean section.

Mortality rates were studied for all infants delivered with a birth weight of 500 gm or more in both periods. Perinatal mortality improved between the periods, especially when like populations were compared excluding the antenatal referrals from the later group (Table VI). There was little change in fetal mortality. Improved results were primarily in the infants of ≥29 weeks, among whom deaths from causes other than lethal anomalies fell from 27 to 2 per 1000 births.

Cause of death was analyzed separately for borderline-viable infants and those of ≥29 weeks (Table VII). Respiratory distress syndrome accounted for most of the decrease in mortality for infants of ≥29 weeks. Twin-to-twin transfusion and necrotizing enterocolitis were each responsible for several deaths in the later period. Neither birth asphyxia nor trauma was the primary cause of death in any infant of ≥29 weeks, including 717 twins delivered vaginally and 169 who had malpresentations.

An attempt was made to determine whether delivery-related factors might have contributed to neonatal death, especially in vaginal births of malpresentations. In both periods 19 neonatal deaths occurred at or after 29 weeks' gestation of causes other than anomalies. The presentation was breech in 10 cases and vertex in nine; in only two of the 10 breech presentations was there severe depression at birth. There was a predominance of second twins (14 compared to five) among the deaths, chiefly because nine of 11 deaths from respiratory distress syndrome occurred in second twins. There was little evidence therefore to suggest that birth asphyxia and trauma played a significant role in the deaths of twins.

### Comment

The increased perinatal mortality associated with twin deliveries is well documented in the literature.<sup>1-4</sup> Although the majority of authors acknowledge that this is due to premature delivery and its attendant complications, great concern regarding birth asphyxia persists. This concern is focused largely on the twin being delivered with an abnormal presentation or very premature twins, for whom cesarean section delivery is often prescribed.

Birth asphyxia in this study has been defined as depression at birth necessitating positive-pressure man-

ual ventilation. In 1978 to 1984 the equivalent mean Apgar scores at 1 minute were 7.6, 4.3, and 2.4, and at 5 minutes 9.1, 7.8, and 5.3, respectively, for infants who required no ventilation, ventilation for <4 minutes, or ventilation for  $\geq 4$  minutes.

No prospective controlled trial has been made of the efficacy of cesarean section in reducing birth asphyxia, trauma, and perinatal mortality in twins. To date, attempts to study the question have been based on comparisons of results in infants delivered vaginally or by cesarean section over similar time periods or by demonstration of changes in outcome from an earlier (mostly vaginal delivery) to a later (mostly cesarean delivery) period. Some of these studies have shown some advantage of cesarean delivery for twins with malpresentations,<sup>1, 3, 4, 8, 9</sup> for second twins with malpresentations,<sup>8, 9</sup> or for twins of very low birth weight.<sup>1, 10, 11</sup> Other authors have doubted that cesarean section is necessary for all twins with malpresentations<sup>1, 2, 8, 11-14</sup>; "This trend is neither supported nor based on recent data."<sup>13</sup>

One reason for the belief that twin morbidity and mortality is related to birth asphyxia is the greater risk of respiratory distress syndrome and death in the second twin.<sup>15-18</sup> This twin is also at greater risk of having a depressed condition at birth. Kenny et al.<sup>16</sup> have shown, however, that the propensity for the second twin to develop respiratory distress syndrome is not related to asphyxia as measured by cord blood pH. It is possible that factors other than asphyxia, such as increased physical manipulation during delivery of the second twin, may be responsible for both the increased depression at birth and the frequent development of respiratory distress syndrome.<sup>16</sup>

This study confirms the higher risk of depression and mortality (especially from respiratory distress syndrome) in the second twin. However, only rarely are infants who die severely asphyxiated at birth, and no cases of postasphyxial encephalopathy or death occurred in this large number of twins delivered vaginally after 28 weeks' gestation.

There is no reduction in the overall incidence of either moderate or severe depression at birth when cesarean section is used liberally. General anesthesia employed in many cesarean sections is associated with depression at birth, as Kelsick and Minkoff<sup>8</sup> suspected.

The lower mortality among twins in recent years is shown in this study to be the result of a marked reduction in neonatal deaths from respiratory distress syndrome and not to reduced incidence or severity of birth asphyxia and trauma. Further reduction in twin mortality will require prevention of extreme prematurity, prevention of respiratory distress syndrome, and early diagnosis and delivery of twins (if viable) who are severely growth retarded or suffering from twin-to-

twin transfusion. Our study has found no justification for the high rates of cesarean delivery of twins which have occurred in this and many other centers in recent years.

Robert Funnell of the Biomedical Engineering Unit, McGill University, was responsible for the development of the perinatal data base of the Royal Victoria Hospital from which the data presented in this publication have been obtained.

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# “Ultrasound rescue”: A successful alternative form of oocyte recovery in patients with periovarian adhesions

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Oocyte recoveries for in vitro fertilization/embryo transfer were performed in 82 cycles in 73 women. The status of the ovaries was unknown. Laparoscopy was performed and oocytes from accessible follicles aspirated. The remaining follicles were aspirated ultrasonographically. The recovery rates for laparoscopy of accessible follicles and for ultrasonographic recovery from laparoscopically inaccessible follicles were identical. In 26 patients laparoscopy only was performed. One or more oocytes was obtained in 92% of patients. In 56 cycles when laparoscopy was followed by ultrasound, one or more oocytes were recovered in 95% of patients; in 12 of these patients, three of whom achieved pregnancy, the only oocytes were recovered by the ultrasonographic means after laparoscopy had failed. This method provided an alternative to screening laparoscopy and indicated that cycles of controlled hyperstimulation could be performed with a satisfactory expectation of oocyte recovery in women in whom the state of the pelvis was unknown. (AM J OBSTET GYNECOL 1986;154:240-4.)

**Key words:** Laparoscopy, ultrasound, oocyte recovery, invitro fertilization/embryo transfer

Preovulatory oocytes for in vitro fertilization and embryo transfer can be obtained by laparoscopic follicular aspiration<sup>1</sup> or by aspiration with use of ultrasonographically directed needles.<sup>2</sup> Although the degree of periovarian adhesive disease does not appear to hinder the ultrasound approach, the ovaries must be accessible to laparoscopy. It has been proposed that the patient should undergo a screening laparoscopy to determine the degree of ovarian accessibility. Wentz et al.<sup>3</sup> attempted to combine screening laparoscopy and timed follicle aspiration during spontaneous ovulatory cycles but were less than satisfied with the results.

Although the ultrasonographic technique may prove to be the method of oocyte recovery of choice, it is probable that many in vitro fertilization/embryo transfer programs will lack the necessary expertise or will have a very limited number of trained personnel. In the initial phases of such programs the laparoscopic method will be employed. In our program although one physician had the necessary technical skills to perform ultrasound oocyte recovery, there were two fully trained laparoscopists. The purposes of this study were

(1) to determine the feasibility of oocyte recovery by a combination of laparoscopy followed by immediate ultrasonographic needle-guided aspiration in a group of unscreened women who were known to suffer from extensive tubal and periovarian disease and who had undergone ovarian stimulation and (2) to compare the efficacy of laparoscopy with ultrasound oocyte recovery in relation to the degree of ovarian accessibility.

The ability to recover oocytes ultrasonographically from laparoscopically inaccessible ovaries was described as “ultrasound rescue.” If this technique proved to be feasible, then the need for preliminary screening laparoscopy would be removed and also it would be possible to identify those patients in whom laparoscopy could be used with ease in any subsequent invitro fertilization/embryo transfer attempts, those in whom ultrasound would be the approach of choice, and those in whom the combined approach should be employed.

## Patients and methods

**Patients.** This paper will describe 82 attempted oocyte recoveries in 73 patients known to suffer from extensive irreparable tubal disease. All 73 underwent one attempted oocyte recovery, and nine underwent a second attempt. The study design called for all patients to undergo an initial laparoscopy. If both ovaries were accessible to laparoscopy, all follicles in the right ovary would be aspirated by this method, the laparoscopy discontinued, and the accessible left ovary aspirated by ultrasonographically directed puncture. If only one ovary was accessible to laparoscopy irrespective of its side, it would be dealt with by this method and the

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remaining inaccessible ovary by ultrasound. If both ovaries were inaccessible, the laparoscope would be removed and both ovaries aspirated with use of the ultrasound method. The nine second attempts were performed in patients who were specifically designated as being most suitable for a combined procedure.

This design was given approval by the Joint Ethics Committee of the University of Calgary and Foothills Hospital. All patients gave informed consent.

**Stimulation.** Ovarian stimulation was carried out with use of clomiphene citrate, 50 mg from day 2 to 6 of the menstrual cycle inclusive. Human menopausal gonadotrophin (Pergonal), 1 ampule (75 IU), was given intramuscularly daily from day 3 to 6 inclusive. Daily estradiol measurements were performed at 8 AM from day 6. If the estradiol measurement had failed to double, then two ampules of Pergonal were given at 3:30 PM. If the estradiol measurement had doubled or was  $>3000$  pmol/L, only one ampule of Pergonal was given. Ultrasonographic follicular tracking commenced on the ninth day of the cycle. When the leading follicle attained a mean diameter (three measurements) of 18 mm, Pergonal was discontinued, and 5000 U of human chorionic gonadotropin was administered 30 to 54 hours later. Oocyte recovery was performed 34 hours after the administration of human chorionic gonadotropin. Four times daily sampling was carried out for luteinizing hormone measurements once the leading follicle had attained a diameter of 15 mm. If the luteinizing hormone attained a value of 180% above the baseline of the mean of the four preceding values, this point was designated to be the luteinizing hormone surge initiating rise<sup>4</sup> and oocyte recovery performed 32 hours from this time.

**Oocyte recovery.** The patient was anesthetized in the dorsal supine position and placed in the frogleg position. The abdomen and perineum were cleansed with antiseptic solution. A Foley catheter that previously had been attached to 1000 ml of saline solution containing dilute methylene blue was inserted in the bladder, and the legs were reextended. The abdomen was draped, pneumoperitoneum was induced with carbon dioxide, and laparoscopy was performed. With the telescope inserted, a second puncture was made suprapubically in the midline and grasping forceps inserted. The patient was placed in a steep head-down position, and the bowel was manipulated gently from the pelvic cavity with the grasping forceps. Each ovary in turn was grasped and inspected to determine its accessibility.

The aspiration system used for both laparoscopic and ultrasound oocyte retrieval was identical. A 22.5 cm stainless steel needle with a 1.5 mm outer diameter and 1.3 mm inner diameter was used. The needle was silicized (Sigma coat, Sigma Chemical Company, St. Louis, Missouri). The needle was connected to a sterile

oocyte trap, suction being provided by the Monash pattern follicular aspiration pump (Cook Surgical, Melbourne, Australia). Aspiration pressures were set at 125 mm of mercury.

Once the follicles had been visualized, the ovary was stabilized with the grasping forceps, and the needle was introduced directly through the abdominal wall. No introducing trocar and cannula were employed. Once the needle tip was visualized within the abdominal cavity, it was flushed by connection of the inflow channel to a syringe containing oocyte culture medium. This flushing effectively dislodged any fat that had collected in the needle tip during its passage through the abdominal wall. Once the surgeon was assured that the needle tip was clean, it was inserted gently into the first accessible follicle and the contents aspirated. Each follicle was flushed with culture medium to a maximum of 10 flushes until either an oocyte was identified or the procedure was abandoned. The other accessible follicles were aspirated by this method.

Once those follicles selected for laparoscopic aspiration had been dealt with, the needle was removed, the grasping forceps and second puncture cannula removed, and the abdomen inspected to exclude evidence of damage. The patient was returned to the horizontal position, the telescope removed, and the gas expelled by opening of the valve of the laparoscopic cannula and exertion of gentle abdominal pressure.

The patient was placed in a 20° reverse-Trendelenburg position, and the bladder filling was started with the opening of the tap of the previously connected system. Sterile ultrasound coupling gel was smeared on the suprapubic area. A 3.5 MHz real-time sector scanner encased in a sterile rubber sheathing was used to monitor the degree of bladder filling and the presence of remaining follicles. A sterile needle guide was clamped to the side of the sector scanner, and a needle path template was placed on the monitor screen. The needle was introduced through the needle guide and its direction defined by reference to the needle path template. The abdominal wall and anterior wall of the bladder were pierced with a stabbing motion. Once the needle tip was identified within the bladder, the system was flushed with culture medium. Slight positive pressure was maintained with the flushing syringe to prevent the entry of any bladder contents to the needle. The needle tip was then advanced into the first accessible follicle and aspiration and flushing performed as previously described. When all follicles had been aspirated, the needle was removed. The bladder was emptied through the catheter and any obvious signs of hematuria noted. The patient was returned to the recovery room.

In all patients the number of follicles punctured and the number of oocytes recovered was recorded.

**Table I.** Oocyte recovery rates by method of aspiration

	Mean number of oocytes per patient	One or more oocytes (%)	Two or more oocytes (%)
Laparoscopy alone (n = 26)	2.8	92	69
Laparoscopy plus ultrasound (n = 56)	3.3	95	84

**Table II.** Recovery of oocytes by method used and ovarian accessibility

	Ovaries accessible		Ovaries inaccessible	
	n	%	n	%
Laparoscopy	124/173	72	0/4	
Ultrasound	27/53	51	76/118	64

Twenty-five patients underwent unscreened first attempts to retrieve oocytes by laparoscopic methods alone, and one patient underwent only laparoscopy during a second attempt. In 14 of these cases the ultrasonographer was not available. In six cases, only one accessible ovary was present; in six patients it was elected to aspirate both ovaries by laparoscopic means because of time constraints in the operating room. Forty-eight first attempts and eight repeat attempts were made with use of a combination of laparoscopy and ultrasound. It was thus possible to compare the efficacy of laparoscopy versus laparoscopy plus ultrasound in a group of 48 unscreened patients and in eight women for whom the combined procedure had been selected as the optimum method for the second attempt.

In addition, it was possible to compare the efficacy of the laparoscopic and ultrasonographic methods of oocyte recovery with respect to the degree of ovarian accessibility.

**Fertilization.** The oocyte culture, sperm preparation, fertilization, and embryo culture methods have been described previously.<sup>5,6</sup> According to the guidelines of our ethics committee, a maximum of only three oocytes per patient may be fertilized and any healthy embryos resulting must be transferred to the patient. Embryo transfer was performed 42 to 48 hours after insemination with use of the Monash transfer catheter as previously described.

If pregnancies were thought to have occurred, they were confirmed by the ultrasonographic detection of the fetal heart by the seventh week of gestation. Any pregnancies so confirmed were correlated to the method of oocyte recovery.

**Statistical methods.** Statistical comparisons were made by means of the  $\chi^2$  test.

## Results

The efficacy of laparoscopy alone as compared with laparoscopy combined with ultrasound in a group of unscreened patients is shown in Table I. In two of the 26 patients who underwent laparoscopy alone no oocytes were recovered. No oocytes were obtained in three of the 56 cycles in which the combined approach was undertaken.

However, multiple oocytes were recovered in 84% of those who underwent the combined procedure compared to only 69% in those who underwent laparoscopy alone.

Not only was it possible to improve the numbers of oocytes recovered by ultrasound rescue, but in 15 of the 56 patients who underwent this method, no oocytes could be recovered by laparoscopy. In three of these 15 patients no oocytes were recovered, in two patients one oocyte was recovered, and in three patients two oocytes were recovered. In three patients there were three oocytes and in an additional further three, four oocytes. Six oocytes were "rescued" in one patient. Thus if all 82 cycles had been managed by laparoscopy alone, at least one oocyte would have been obtained in only 62 cycles (76%).

Table II demonstrates the number of oocytes recovered per follicle punctured by either the laparoscopic or ultrasonographic method, depending on whether the ovaries were judged to be accessible to laparoscopy. Although the ultrasound method was less successful than laparoscopy if the ovaries were accessible (51% versus 72%,  $p < 0.001$ ), the success rates when laparoscopic aspiration of accessible follicles were compared with ultrasonographic aspiration of inaccessible follicles were similar (72% versus 64%,  $p > 0.1$ ). The overall ultrasound recovery rate (accessible plus inaccessible follicles) was 60% (103 of 171 follicles) and was less ( $p < 0.025$ ) than the 72% (124 of 173 follicles) rate of laparoscopic recovery from accessible follicles.

There were two complications. Two patients complained of transient hematuria, which resolved within 2 hours and 4 hours, respectively. Ten pregnancies were confirmed. Of these 10 pregnancies the oocytes had been recovered by laparoscopy alone in four cases and by a combination of ultrasound and laparoscopy in three, and three occurred in the ultrasound rescue group. The pregnancy rate per attempted oocyte recovery therefore was 12%.

Because it is not possible for our ultrasonographer to be present for every oocyte recovery, it would be logical that patients requiring second attempts at oocyte recovery should be designated as those who would re-

quire ultrasonographic methods alone, those requiring laparoscopy alone, or those who might be considered for a second laparoscopy combined with ultrasonography. Of the 73 patients who underwent first recovery attempts 27 were rescheduled for ultrasound alone, 28 for laparoscopy alone, and 18 for a combination of both procedures for any subsequently required oocyte recovery.

### Comment

Laparoscopic oocyte recovery for in vitro fertilization/embryo transfer can only be performed if the ovaries can be visualized at the time of laparoscopy. In an early report<sup>7</sup> of 853 laparoscopies 449 were carried out as assessment procedures. During these assessment procedures laparoscopic adhesiolysis was performed when necessary, and in 55 other cases immediate laparotomy was required to effect adhesiolysis. The demonstration by Lenz and Lauritsen<sup>2</sup> that oocytes could be recovered by means of ultrasonographically guided needles and that this procedure was not hampered by severe pelvic adhesive disease has offered an alternative approach to oocyte recovery in such patients.

Wentz et al.<sup>3</sup> attempted to combine a screening laparoscopy, by which ovarian accessibility could be determined, with timed follicle aspiration in spontaneous cycles in which human chorionic gonadotropin was administered once the follicle had reached a mean diameter of >15 mm, the cervical mucus was highly estrogenic, and the cervical os was dilated. The cycle length was taken into consideration. By this approach they were able to assess the accessibility of the ovaries but were only able to collect a mean number of 0.73 eggs per patient; they decided that the procedure offered little ultimate benefit to the patient.

Wikland et al.<sup>8</sup> have clearly demonstrated the feasibility of human oocyte recovery with use of ultrasonography and obtained oocytes from 77% of follicles so punctured. Indeed when the recovery rate was corrected for the presence of cysts (defined as follicles >26 mm in diameter) the recovery rate was 87% per follicle.

This study was conducted because of limited numbers of personnel with ultrasonographic skill and a desire to avoid preliminary laparoscopy or surgical intervention. A secondary objective was to compare our efficiency in collecting oocytes by both the laparoscopic and ultrasonographic methods. Although Wikland et al.<sup>8</sup> have demonstrated that it is possible in some patients to recover oocytes by laparoscopy after an initial attempt at oocyte recovery has failed, it was felt that in unscreened patients this approach would have certain disadvantages. It is not possible to determine by ultrasonography whether the ovaries are covered with adhesions and whether, if subsequent laparoscopy is

performed, only accessible follicles can be punctured. If laparoscopy is performed first, accessible follicles can be dealt with, leaving those inaccessible to be punctured by the ultrasonographic method. A possible disadvantage of this latter approach is that any residual carbon dioxide left within the abdomen might compromise ovarian and follicular visualization by ultrasound. Our experience has been that although the ultrasonographic image is somewhat less clear, this approach does not preclude successful oocyte retrieval.

Lenz and Lauritsen<sup>2</sup> have suggested that by fixing the ovary, periovarian adhesions might make it easier to recover oocytes than if the ovary were freely mobile. We were able to obtain oocytes in only 51% of follicles in mobile ovaries by the ultrasonographic method but in 64% of follicles when the ovaries were fixed; while not statistically significant ( $p > 0.05$ ) this observation would tend clinically to support Lenz's and Lauritsen's contention. From these data it would appear that our ability to recover oocytes by laparoscopy from accessible ovaries was comparable to our ability to recover oocytes by ultrasonography when the ovaries were inaccessible. An overall ultrasonographic oocyte recovery (accessible plus inaccessible ovaries) rate of 60% compares well with the 58% reported by Lenz and Lauritsen<sup>2</sup> but less favorably with the corrected 87% reported by Wikland et al.<sup>8</sup>; it was also less effective ( $p < 0.025$ ) than our ability to obtain oocytes by laparoscopy from accessible follicles.

When those patients who were treated by laparoscopy alone were compared with those patients for whom ultrasound rescue was attempted, in the former group one or more oocytes were recovered in 92% of patients and multiple oocytes in only 69%. In the latter group at least one oocyte was recovered in all but 5% and multiple oocytes in 84% of patients. Included in this group were 12 women in whom oocyte recovery would have been a complete failure had not the ultrasound backup been available; three pregnancies were achieved in these 12 women.

Only two complications (transient hematuria) were attributable to the ultrasound method, which is in keeping with the incidence of two cases of hematuria noted in 44 cases by Wikland et al.<sup>8</sup>

By using preliminary laparoscopy we were able to recover oocytes satisfactorily from patients with accessible ovaries by a method with which the surgeons were familiar. Although the single ultrasonographer had to try to be present for every first attempt, it was possible by use of this approach to carry out a screening laparoscopy and simultaneous oocyte recovery so that second attempts could be designated as laparoscopy, ultrasound, or combination, thus reducing the load on ultrasound services for second and subsequent oocyte



recoveries while ensuring their availability in laparoscopically impossible cases. The experience gained from this work had demonstrated clearly that if this approach is to be used during the first cycle, the ultrasonographer must always be present.

Although it is possible that oocyte recovery by ultrasonography may become the method of the future, thus replacing the need for screening laparoscopy, it is probable that many programs will continue to use laparoscopy for some time to come. If the technique of ultrasound rescue is employed, it is possible to remove the need for screening laparoscopy and more importantly prior laparotomy and salpingoovariolysis without compromising the ability to recover oocytes and achieve pregnancies by in vitro fertilization/embryo transfer.

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## Has use of cesarean section reduced the risks of delivery in the preterm breech presentation?

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The effect of cesarean section on the condition at birth in cases of preterm breech presentation was studied in consecutively delivered infants in two time periods. Delivery was rarely (8%) by cesarean section in 1961 to 1974 and usually (89%) by cesarean section in 1978 to 1984. The increased cesarean rate did not reduce the incidence of severe depression, which was double that in control cases with cephalic presentations in both periods. Breech births did not have a higher mortality rate than cephalic births in either period; birth trauma and encephalopathy were similar in both periods. Cesarean section was therefore not found to reduce either the incidence of depression at birth or the mortality. However, head entrapment was responsible for the deaths of seven of 55 live-born infants delivered vaginally at 25 to 28 weeks' gestation, all weighing <1000 gm. Although cesarean section is at present performed least often among these extremely premature infants, it is in these cases that it may prove most beneficial. (*AM J OBSTET GYNECOL* 1986;154:244-50.)

**Key words:** Preterm breech presentation, cesarean section, birth asphyxia, perinatal mortality

Over the past decade there has been a marked increase in frequency of use of cesarean section for

breech presentations. In a previous report from this hospital the effect of such a change in management on the outcome of infants with breech presentation who were delivered at term was minimal; the incidence of birth asphyxia was unchanged.<sup>1</sup> Vaginal breech delivery may, however, represent a greater hazard to the preterm infant, both because of the inherent vulnerability of a more fragile infant and because of the greater relative discrepancy between head and body size.

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**Table I.** Distribution of study populations by gestational age

Gestational age (wk)	1961-1974		1978-1984	
	% of Breech births— group A* (n = 170)	% of Cephalic births— group B (n = 1864)	% of Breech births— group C (n = 116)	% of Cephalic births— group D (n = 903)
25-28	22	6	16	5
29-32	21	11	25	14
33-36	57	83	59	81
Total	100	100	100	100

\*Standard gestational age distribution for adjustments.

**Table II.** Incidence of cesarean section by gestational age

Gestational age (wk)	Breech*		Cephalic*	
	1961-1974 (n = 170)	1978-1984 (n = 116)	1961-1974 (n = 1864)	1978-1984 (n = 903)
25-28	3	44	1	7
29-32	3	97	8	19
33-36	12	97	5	14
Total	8	89	5	14

\*Percent of infants at each gestational age who delivered by cesarean section.

Assessing the impact of cesarean birth on preterm breech presentation is complicated by the marked improvement in perinatal care that has occurred concurrent with the increase in cesarean section rate. This study compares the relative risks of delivery-related morbidity and mortality in breech births compared to those of cephalic births in control cases before and after cesarean section became the preferred method of preterm breech delivery in this hospital.

### Population and methods

The infants studied were preterm singletons who were delivered in the Royal Victoria Hospital before (1961 to 1974) and after (1978 to 1984) cesarean section became the preferred method for breech delivery. The population included infants of 25 to 36 completed weeks, of birth weight appropriate for gestational age,<sup>2</sup> weighing at least 500 gm, excluding cases with abruptio placentae, Rh hemolytic disease, toxoplasmosis, rubella, cytomegalic inclusion disease, and herpes simplex virus (TORCH) infections, and lethal fetal malformations.

To control for differences in gestational age distribution between breech and cephalic presentation, the populations were subdivided into three 4-week gestational age groups for comparison of infants within similar gestational age strata (Table I). To determine statistical probability of differences observed within each gestational interval,  $\chi^2$  tests were used. The Mantel-Haenszel formula was used to test whether the differences found in the three gestational strata were statistically significant when combined.

The breech/cephalic index of depression at birth and the mortality were compared for all preterm infants in the two time frames. This was done before and after the gestational age distribution of each group of infants was adjusted to that found in the "standard" group A (Table I).

Maternal and infant diagnoses were coded at the time of discharge and entered into the computer. All neonatal records were reviewed and diagnoses standardized with use of the same criteria throughout the two periods by one of us (R. U.), who was also responsible for the care of most of the infants. Deaths were reviewed at time of occurrence by a committee, and primary cause assigned with use of the same classification system and criteria throughout both periods. Autopsy rates exceeded 90%.

Outcome variables consisted of (1) severe depression at birth, defined by the need for positive-pressure ventilation for more than 3 minutes after birth to establish sustained spontaneous respirations; (2) postasphyxial encephalopathy, defined as abnormal cerebral depression, irritation, or convulsions caused by peripartum asphyxia; (3) fractures or peripheral nerve palsies persisting to discharge; and (4) neonatal deaths at any age before discharge to the home, including those occurring after transfer to another hospital for surgery, etc.

Charts for mother and neonate for each case of breech presentation delivered at or after 29 weeks and presenting one or more of the above abnormalities were reviewed by one of us (B. B.) to determine factors contributing to the poor outcomes, particularly those that might be related to breech presentation.

**Table III.** Incidence of severe depression at birth\*

Gestational age (wk)	Time period	Breech		Cephalic		Index (breech/cephalic)	p
		No.	%	No.	%		
25-28	1961-1974	18/37	48.6	27/103	26.2	1.9	<0.02
	1978-1984	11/18	61.1	16/44	36.4	1.7	NS
29-32	1961-1974	4/36	11.1	17/213	8.0	1.4	NS
	1978-1984	8/29	27.6	10/131	7.6	3.6	<0.01
33-36	1961-1974	5/97	5.2	14/1548	0.9	5.8	<0.01
	1978-1984	8/69	11.6	10/728	1.4	8.3	<0.001
Total 25-36	1961-1974	27/170	15.9	58/1864	3.1	5.1	
	1978-1984	27/116	23.3	36/903	4.0	5.8	
Adjusted total† 25-36	1961-1974	27/170	15.9	147/1864	7.9	2.0	
	1978-1984	30/116	26.0	92/903	10.2	2.5	

\*When the  $\chi^2$  values for the three gestational age strata (Mantel-Haenszel expression) were combined, the rate of severe depression was significantly higher ( $p < 0.001$ ) among breech than among cephalic births in both 1961 to 1974 and 1978 to 1984.

†After gestational age distribution was adjusted to that of the standard population (see Population and methods).

**Table IV.** Incidence of severe depression by type of anesthetic (cesarean deliveries at 29 to 36 weeks, 1978 to 1984)

	General anesthetic		Epidural anesthetic		p
	No. delivered	% depressed	No. delivered	% depressed	
Breech	44	25.0	51	7.8	<0.025
Cephalic	62	16.1	62	0	<0.001

## Results

The cesarean section rate for preterm breech presentations rose from 8% in the early period to 89% in the later. In cases with cephalic presentations the increase was much more modest (Table II).

Severe depression at birth was five times more frequent in breech than in cephalic births in the early period (Table III). There was no reduction in this breech/cephalic index of severe depression in the later period. A large part of the increased risk of severe depression at birth in breech presentations was due to the lesser maturity of infants in breech presentations. After adjustment for differences in gestational age, however, a twofold greater risk of severe depression among breech infants existed. Severe depression occurred no less frequently during the later period in infants born breech or cephalic; instead it tended to increase.

The increased incidence of severe depression in the later period was partly related to the depressant effect of general anesthesia used for one half of the cesarean sections. The data in Table IV show that severe depression occurred much more frequently when cesarean section was done under general rather than conduction anesthetics. When only those cesarean sections done with epidural anesthetic are considered, the rate of severe depression among infants with breech presenta-

tions in weeks 29 to 36 in 1978 to 1984 was 7.8% (four of 51). This rate was similar to the 6.8% rate (nine of 133) found in the years 1961 to 1974 when almost all breech presentations were delivered vaginally (Table III), indicating that difficulties in breech delivery occurred in cesarean as well as vaginal birth.

The most severely asphyxiated newborn infants who survive usually manifest signs of postasphyctic encephalopathy (tonic/clonic convulsions in the worst cases) during the first week of life. Such signs developed in six of 133 breech presentations with delivery at 29 to 36 weeks in the early period (4.5%) compared to four of 98 (4.1%) in the later period. Two cases in the early and one in the later period developed convulsions.

Peripheral nerve injury occurred in two infants with breech presentations in each period; all four had brachial plexus palsy and one an additional facial palsy. There were no fractures.

The factors contributing to severe depression, encephalopathy, and birth trauma in breech birth are tabulated in Table V. In the early time period affected infants were all delivered vaginally. In no cases did mechanical difficulty at delivery contribute to abnormal outcome. Prolapsed cord and intrauterine infection contributed to the poor outcome in some cases. Forceps to the aftercoming head were employed in some a-



**Table V.** Causative factors in breech deliveries with abnormal outcome, 29 to 36 weeks

	No. of cases	No. of cesarean deliveries	Forceps to aftercoming head	Difficult cesarean delivery	Prolapsed cord	Infection in utero	General anesthesia	Oligohydramnios	No causative factors
Severe depression									
1961-1974	10	0	4	0	3	2	0	0	3
1978-1984	16	15	0	5	0	5	11	4	0
Encephalopathy									
1961-1974	6	0	4	0	2	0	0	0	2
1978-1984	4	3	0	2	0	1	1	0	0
Fractures/palsies									
1961-1974	2	0	2	0	1	0	0	0	0
1978-1984	2	1	0	1	0	0	0	0	1

**Table VI.** Neonatal mortality rate\*

Gestational age (wk)	Time period	Breech		Cephalic		Index (breech/cephalic)	p
		No.	%	No.	%		
25-28	1961-1974	26/37	70.3	61/103	59.2	1.2	NS
	1978-1984	9/18	50.0	18/44	40.9	1.2	NS
29-32	1961-1974	3/36	8.3	28/213	13.1	0.6	NS
	1978-1984	2/29	6.9	2/131	1.5	4.6	NS
33-36	1961-1974	2/97	2.1	17/1548	1.1	1.9	NS
	1978-1984	0/69	0	3/728	0.4	0	NS
Total 25-36	1961-1974	31/170	18.2	106/1864	5.7	3.2	
	1978-1984	11/116	9.5	23/903	2.5	3.8	
Adjusted total† 25-36	1961-1974	31/170	18.2	300/1864	16.1	1.13	
	1978-1984	14/116	12.1	84/903	9.3	1.30	

\*When the  $\chi^2$  values for the three gestational age strata (Mantel-Haenszel expression) were combined, the neonatal mortality rate was not significantly higher among breech than among cephalic births either in 1961 to 1974 or in 1978 to 1984.

†After gestational age distribution was adjusted to that of the standard population (see Population and methods).

fects infants and may have contributed to asphyxia or trauma. They were seldom used in this study except after 32 weeks of gestation. The incidence of severe depression was two of 43 (4.7%) in cases of 33 to 36 weeks' gestation without use of forceps, and three of 28 (10.8%) when forceps were used (NS).

During the more recent period almost all infants with abnormal outcomes were delivered by cesarean section. The uterine incision did not ensure easy breech extraction, because serious mechanical difficulty was encountered in almost one half of the infants with poor outcomes. Oligohydramnios was often a factor contributing to difficult cesarean birth. General anesthesia during cesarean section and intrauterine infection were additional factors causing depression at birth.

Frank and footling breech presentations in deliveries in the early (vaginal) period had similar rates of severe depression: 19% of 27 frank breech and 22% of 85 footling breech presentations in which type of breech was specified. All cases of prolapsed cord occurred in footling breech presentations.

Prolapse of the umbilical cord presents a particular risk of preterm breech delivery. It occurred in 16 in-

fants (9.4%) in the early (vaginal delivery) period and in three infants (2.6%) in the later (cesarean section) period. Among the 13 cases of prolapsed cord after 28 weeks, in only three was the infant severely depressed at birth. There was one death associated with but not caused by a prolapsed cord: a nondepressed infant who died of respiratory distress syndrome.

Neonatal mortality was 3.2 times greater in breech than in cephalic births in the early period (Table VI). When the gestational age distribution of cephalic infants was adjusted to that found in the breech infants, however, the relative mortality risk of being a breech compared to cephalic was negligible (1.1). Within each gestational age category and when data from all three categories were analyzed together, there was no significant difference between neonatal mortality in breech and cephalic births at a time when breeches were being delivered vaginally.

In the later period neonatal mortality in breech infants decreased. However, there was a similar decrease in mortality among cephalic infants such that there remained a 3.8 times greater risk of mortality among breech births in the more recent time period. Once

Table VII. Neonatal deaths, 29 to 36 weeks

Birth weight (gm)	Gestational age (wk)	Type of delivery	Depression at birth	Age at death	Cause of death	Comments
1961-1974						
1340	30	Breech, precipitate	None	3 hr	Respiratory distress syndrome	Bicarbonate bolus, intraventricular hemorrhage
1400	33	Breech, spontaneous	Severe	24 hr	Respiratory distress syndrome	Depression of unknown cause at birth
1655	32	Breech, assisted, easy	Moderate	6 hr	Meconium peritonitis	Severe respiratory distress syndrome
1100	30	Breech, assisted, easy	Severe	½ hr	I.U. pneumonia	Ruptured membranes 7 days earlier; chorioamnionitis
2100	36	Breech, precipitate	Moderate	32 hr	Respiratory distress syndrome	Prolapsed cord
1978-1984						
1460	30	Breech, difficult cesarean section	Severe	5 mo	Necrotizing enterocolitis	Did well from day 4 until day 20 when septicemia developed
1790	32	Breech, assisted	Severe	6 days	Asphyxia	Encephalopathy, intraventricular hemorrhage

again, the breech/cephalic mortality risk index dropped after adjustment for differences in gestational age distribution to 1.3.

These data show that after adjusting for gestational age differences, preterm infants with breech presentation delivered vaginally in the early time period were not at higher risk of dying than infants with cephalic presentations. The improvement in mortality seen in recent years among breech births is no greater than the improvement among cephalic births, in spite of a six-fold higher cesarean rate for breech presentations.

The seven infant deaths among the 211 preterm breech presentations delivered at 29 to 36 weeks are described in Table VII. Among 120 vaginal breech deliveries during the early period, none of the five deaths were due to difficult breech births. Two infants who died were severely depressed at birth, one from intrauterine infection. There were three deaths from respiratory distress syndrome. Both deaths in the later period were of infants who experienced serious difficulty in delivery (one in a vaginal and the other in a cesarean delivery), with delivery-related asphyxia the cause of death in the vaginal birth. This was the only delivery-related breech death after 28 weeks' gestation.

Regarding the infants delivered before 29 weeks' gestation, difficult breech delivery played a larger role in mortality. Among the 37 breech births at 25 to 28 weeks during the early (vaginal) period, 26 died. Five of these deaths, all with weights of <1000 gm, were caused by difficult vaginal breech delivery. The remaining 21 were due to respiratory distress syndrome (6), infection (3), and other complications of prematurity in 12 infants who weighed <1000 gm.

Among 18 infants of 25 to 28 weeks who were delivered in the later period, there were nine deaths. Two were due to difficult vaginal breech delivery, and the remainder were caused by respiratory distress syn-

drome (2), infection (2), and other prematurity-related conditions in infants of <1000 gm (3).

An additional death resulting from asphyxia from difficult vaginal breech birth occurred during the early period in an infant who was excluded from the study because of being small for gestational age (35 weeks, 1100 gm). This was the second death beyond 28 weeks attributable to difficult delivery in a breech birth throughout the study periods. Difficulty in delivery not sufficient to cause death was also experienced in several other small-for-dates infants with oligohydramnios, occurring in both vaginal and cesarean deliveries.

Intrapartum fetal deaths, though excluded from the live-born study groups, were reviewed to determine the possible role of difficult breech delivery or prolapsed cord. There were seven deaths in labor during the early period and one during the later period, all but two occurring before 29 weeks. Intrauterine infection accounted for three of the deaths. Among the remaining five there were two in which breech presentation played a role. One was due to prolapsed cord, and one was due to placenta previa, though complicated by difficult breech delivery with head entrapment; both weighed <1000 gm.

### Comment

The trend in the 1970s toward more liberal use of cesarean delivery for the premature breech presentation reported here is similar to that noted in the literature in Europe<sup>3,4</sup> and North America.<sup>5,6</sup> This change was stimulated by the desire to reduce the risk of breech-related birth asphyxia and trauma.

In the present study the vaginal breech delivery of premature infants was associated with twice the incidence of depression found in cephalic births, a difference also reported by others.<sup>4,7,8</sup> Cesarean birth, however, did not decrease this relative risk. Similar findings

were reported by some previous authors,<sup>4, 9, 10</sup> though others have shown a benefit with cesarean birth in cases of premature breech presentation.<sup>3, 11</sup>

What might be the factors in cesarean delivery that cause as high a depression rate as in vaginal delivery? Use of general anesthesia was associated with a twofold to threefold higher depression rate compared to epidural anesthesia. However, even with use of epidural anesthesia, cesarean sections had similar depression rates as breech presentations delivered vaginally. A second factor may be the type of uterine incision made. In a poorly developed lower uterine segment, such as may be seen with premature footling breech presentations, a low vertical or classical cesarean section may facilitate delivery; however, in a study comparing low transverse with low vertical cesarean sections, no significant differences were seen with respect to perinatal mortality and Apgar score.<sup>12</sup> In the present study the vast majority of cesarean sections were of the low transverse type. Another important factor is the amount of amniotic fluid. In this study oligohydramnios was frequently associated with difficult breech delivery, particularly at cesarean section. If abdominal delivery of breech presentations is to be performed less traumatically, studies will be required to define the optimal type of incision to be made as well as the best anesthetic agent to combine maximal uterine relaxation with minimal depression of the infant.

The factors responsible for depression after vaginal breech birth are somewhat different than expected. Head entrapment was not a significant problem in the present study among infants of 29 to 36 weeks. Though some of the most severely affected infants were delivered by forceps to the aftercoming head, depression at birth was equally frequent when extraction of the head was performed manually. One author has reported a beneficial effect of routine forceps applications to the aftercoming head.<sup>13</sup> Prolapse of the umbilical cord, though frequent with footling breech presentation, rarely produced severe depression. The relative innocuousness of prolapsed cord in the preterm breech has been reported previously,<sup>14</sup> and is presumably related to the timing of the prolapse late in second stage when immediate delivery is feasible.

Fractures and palsies were quite rare in the present study and occurred after both vaginal and cesarean birth.

With respect to mortality, studies in the literature investigating the effect of the route of delivery on the outcome of the premature breech presentation assume that these infants if delivered vaginally are at greater risk of dying than their counterparts with cephalic presentations. Our study does not demonstrate such an increased risk, and the published reports give variable results.

Kauppila et al.<sup>4</sup> found a 2.9-fold increased risk of uncorrected neonatal mortality of infants with premature breech presentation compared to those with cephalic presentation. This finding is partly explained by the inclusion of lethal congenital anomalies, which are more common among breech presentations. Furthermore, most of the breech deaths were among the very small infants. Goldenberg et al.,<sup>7</sup> after excluding antepartum stillbirths and malformations, found only a 1.2- to 1.5-fold increased risk among the 750 to 1500 gm infants and no increased risk among the 1500 to 2500 gm breech infants delivered vaginally. Likewise, Main et al.<sup>9</sup> in a study of newborn infants excluding congenital anomalies found that infants with breech presentation weighing <1500 gm have a 1.4 to 1.6 times higher neonatal mortality than their counterparts with cephalic presentation. In a similar study, after excluding malformations, Smith et al.<sup>8</sup> found a 2.2- and 4.0-fold increase in neonatal mortality among 1000 to 1500 gm and 1500 to 2000 gm infants, respectively.

Since the present study does not demonstrate an increased mortality risk for the infant with premature breech presentation when most deliveries were vaginal, it is not surprising that the sixfold increase in cesarean rate in the later period did not reduce this breech/cephalic mortality risk ratio.

Analysis of the causes of death among infants of 29 to 36 weeks revealed only one vaginal delivery-related death of an infant delivered at 32 weeks by breech extraction. However, among the 55 live-born infants of 25 to 28 weeks' gestation, seven of 35 deaths were attributed to head entrapment during vaginal delivery.

Several reports<sup>4, 6, 10, 13, 16</sup> suggest that infants weighing <1500 gm may benefit the most from an abdominal delivery, and our study supports this contention. Yet it is noteworthy that the cesarean rate has increased the least in this group.

As the survival rate continues to improve, the reluctance to perform a cesarean section for a very premature infant is diminishing. Perhaps an abdominal delivery might further improve the outcome of the very premature breech infants.

In the preterm breech presentation of 29 to 36 weeks' gestation, there is no evidence that a policy of routine cesarean delivery is advantageous.

Robert Funnell of the biomedical Engineering Unit-McGill University has been responsible for the development of the perinatal data base of the Royal Victoria Hospital from which the data presented in this publication have been obtained.

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## Fetal heart rate monitoring and neonatal mortality in the very preterm infant

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A retrospective study was performed to determine the usefulness of intrapartum fetal heart rate patterns in managing infants of 26 to 30 weeks' gestational age by a comparison of intrapartum tracings with neonatal outcome. Fetal heart rate patterns of 26 infants who died were matched for gestational age with those of infants who did not die or demonstrate developmental abnormalities after a 1-year follow-up were analyzed. A normal fetal heart rate pattern was associated with a good outcome ( $p < 0.05$ ), the only deaths (three) being secondary to unrelated factors. An abnormal fetal heart rate tracing predicted 90% of deaths; however, an abnormal fetal heart rate tracing was also found in 15 of 31 infants with no mortality or morbidity. Evidence would thus suggest that the very preterm infant can tolerate the stress associated with normal labor and that a normal fetal heart rate pattern predicts good fetal outcome in the absence of unrelated perinatal abnormality. With significantly abnormal patterns, however, further parameters must be evaluated before the diagnosis of fetal distress associated with subsequent mortality can be made with certainty. (*AM J OBSTET GYNECOL* 1986;154:250-4.)

**Key words:** Intrapartum fetal heart rate monitoring, preterm morbidity/mortality

The cost of supporting the very preterm infant is enormous. However, most recent articles have con-

cluded that pediatric outcome justifies this expense.<sup>1,2</sup> Nonetheless, the management of the very preterm infant in labor is still contentious. Consensus does not even exist as to whether the very preterm infant can tolerate the stress of normal labor. Certainly every effort must be made to avoid fetal asphyxia or trauma and this had led to the liberal use of cesarean section when fetal distress is suspected.<sup>3</sup>

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The use of intrapartum fetal heart rate patterns to diagnose fetal distress has been well studied and reviewed in the term infant. Despite this, the value of routine electronic fetal monitoring is still questionable in terms of neonatal outcome. In contrast, the value of intensive electronic monitoring of the very preterm infant has not been well studied and what data are available are inconclusive. This study was proposed to further define the usefulness of fetal heart rate patterns in managing the very preterm infant, specifically to relate neonatal outcomes in the infant of 26 to 30 weeks' gestational age to first-stage fetal heart rate patterns.

### Material and methods

The study population was taken from all infants born in the Perinatal Unit at Women's College Hospital at an obstetric gestation of <32 weeks between January 1, 1979, and December 31, 1982. In total, there were 730 live-born infants during this time. All infants with congenital anomalies incompatible with life were excluded as were infants born before 26 weeks or after 30 weeks. Exclusion of those infants <26 weeks' gestation was to avoid the possibility that suspected borderline gestations may have compromised the perinatal approach. Exclusion of those infants >30 weeks' gestation was due to incomplete 1-year follow-up. This left a group of 383 live-born infants with gestational ages between 26 and 30 weeks.

In this population, 39 infants subsequently died. Each dead infant was matched to the infant born closest to its birth date with the same gestational age who did not die or demonstrate abnormality after a 1-year follow-up. Of the 39 dead and 39 matched control infants all intrapartum tracings were obtained where possible. A total of 57 tracings were obtained from 26 dead and 31 control fetuses. Obstetric and neonatal variables were recorded and analyzed for each infant and are shown in Table I. Umbilical vein measurements were used as is the routine at this center.

First-stage fetal heart rate patterns were analyzed during the last 30 minutes of first-stage labor if vaginal delivery ensued or for the 30 minutes prior to cesarean section if an abdominal delivery was selected. Tracings were obtained by a combination of indirect and direct electronic signals, the majority being direct.

All tracings were read independently in a blinded fashion to each other and to neonatal outcome by two observers. The first (N. D. J. B.) used the quantitative scoring systems developed by Fischer et al.<sup>4</sup> and Hammacher<sup>5</sup> to define whether a tracing was normal or abnormal with respect to both overall score and individual parameters. If abnormal, the tracing was further subdivided as to whether it displayed an increased baseline of >160 bpm, absent accelerations, decreased variability, or decelerative activity. Decelerative activity

Table I. Variables

Maternal age
Parity
Onset of labor
Premature labor
Premature rupture of the membranes
Antepartum hemorrhage
Singleton gestation
Maternal complications
Vertex presentation
Cesarean section delivery
Cesarean
Anesthetic
Gestational age at delivery
Weight
Appropriate for gestational age—small for gestational age
Apgar scores at 1 and 5 min
Umbilical vein pH
Base deficit
Incidence of respiratory distress syndrome
Intraventricular hemorrhage, 1-2
Intraventricular hemorrhage, >3
FHR pattern
Fischer-Hammacher score

itself was divided into benign variable decelerations or mixed variable and late decelerative activity. Benign variable decelerations were defined by the criteria of Krebs et al.<sup>6</sup> Variability was defined according to criteria of Fischer et al.<sup>4</sup> and Hammacher.<sup>5</sup> Statistical analysis was then carried out with the Student *t* test for numerical data and corrected  $\chi^2$  analysis for incidence, with the quantitatively scored tracings compared with neonatal outcome.

In an attempt to compare quantitative analysis with the widely used qualitative analysis, a second observer (J. E. M.) evaluated each tracing in the standard fashion based on experience used at this center. Agreement between observers was noted in 90% of cases. Good agreement was also found between increasing severity of abnormality by qualitative assessment and decreasing Fischer or increasing Hammacher scores. However, when individual parameters were compared, quantitative analysis proved to be more sensitive in noting minor reductions in variability and increases in baseline. Forty percent of tracings read by qualitative assessment as normal were noted on quantitative assessment to have such minor changes in individual parameters.

### Results

A breakdown of the study group is shown in Fig. 1. Both groups were apparently well randomized for pregnancy, delivery variables (Table II), and neonatal factors (Table III).

Significant differences were noted in neonatal status at birth between the infants who died and the control group. The infants who died had significantly lower 5-minute Apgar scores ( $p < 0.005$ ), lower umbilical ve-

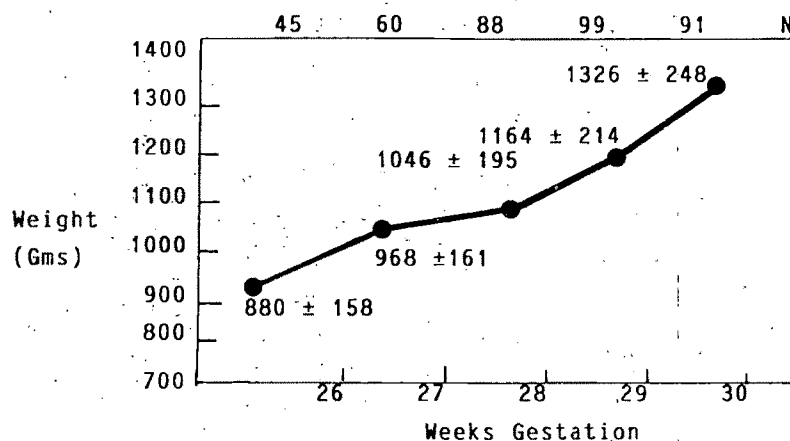


Fig. 1. Number and weights of infants by gestational age.

Table II. Pregnancy-delivery factors

Factors	Dead infants (N = 26)	p	Control infants (N = 31)
Maternal age (yr)	26.3 ± 5.6	NS	25.7 ± 6.0
Premature labor (n)	5	NS	3
Premature rupture of the membranes (n)	13	NS	12
Antepartum hemorrhage (n)	2	NS	5
Vertex presentation (n)	17	NS	18
Cesarean section (n)	19	NS	20
General anesthetic (n)	15	NS	16

Table III. Neonatal factors

Factor	Dead infants (N = 26)	p	Control infants (N = 31)
Gestational age (wk)	27.5 ± 1.5	NS	27.5 ± 1.5
Weight (gm)	960 ± 243.7	NS	1043.9 ± 245.3
Appropriate for gestational age (n)	20		28
Small for gestational age (n)	6		3

nous cord pH values ( $p < 0.05$ ), and higher cord base deficits ( $p < 0.05$ ) when compared with values in their controls (Table IV).

Subsequent neonatal course also demonstrated a higher incidence of significant intraventricular hemorrhage in infants who subsequently died ( $p < 0.005$ ).

Sixty-six percent of all fetal heart rate tracings showed some abnormality. However, the infants who died had significantly poorer Fischer and Hammacher scores when compared with those of their controls (Table V). When an attempt was made to compare individual fetal heart rate patterns with outcome, no statistically significant pattern was found, apart from a normal pattern being associated with a good outcome ( $p < 0.05$ ). Individual abnormal patterns failed to reach significance, possibly because of small sample size

Table IV. Neonatal status

	Dead infants (N = 26)	p	Control infants (N = 31)
Apgar score			
1 min	4.12 ± 2.4	NS	5.0 ± 2.2
5 min	6.7 ± 2.3	<0.005	8.0 ± 1.3
Cord pH	7.26 ± 0.17	<0.05	7.33 ± 0.09
Base deficit	-8.5 ± 6.2	<0.05	-5.2 ± 4.1
Respiratory distress syndrome	20	NS	12
Intraventricular hemorrhage			
1-2	2	NS	5
3-4	15	<0.005	1

or lack of specificity. An abnormal tracing was found in 90% of infants who died, but 50% of infants who survived also displayed some fetal heart rate abnormality. Importantly, only three infants with normal fetal heart rate patterns subsequently died. Two of these infants were delivered because of clinical abruptio placentae at 27 weeks' gestation, and both fetal heart rate patterns had been recorded during early uterine activity before cesarean section. One of these infants died secondary to pulmonary hypoplasia. The second died of bilateral pneumothorax. Both were found to have extensive lung disease incompatible with extrauterine life. The third infant died after delivery by cesarean section for placenta previa complicated by premature rupture of the membranes and chorioamnionitis at 27 weeks. When an attempt was made to compare types of fetal heart rate abnormalities with Apgar scores and pH values, only cord venous pH values were found to be significantly different if one assessed individual parameters alone (Table VI). Both decreased variability and severe decelerative tracings were associated with a lower cord venous pH value. A trend to a lower 5-minute Apgar value was also noted in infants displaying severe decelerative tracings. No significant difference was noted when tracings exhibited baseline increases



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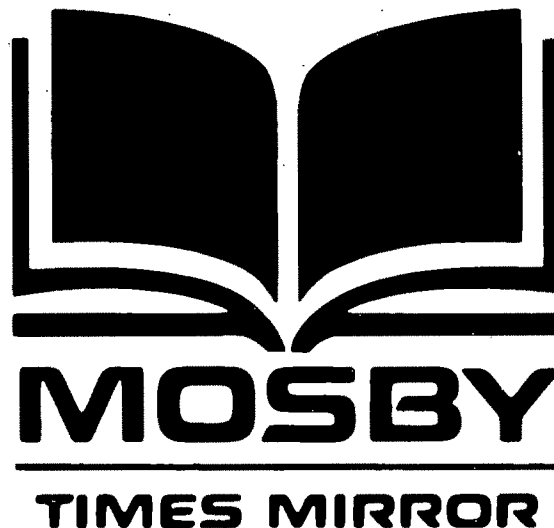
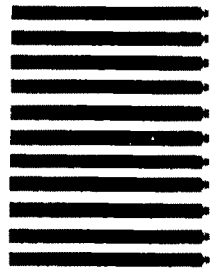
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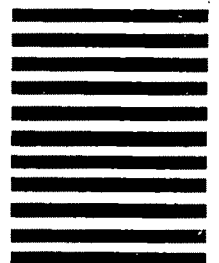
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**Table V.** Fetal heart rate tracing versus neonatal outcome

	Dead infants (N = 26)	p	Control infants (N = 31)
Fischer	6.6 ± 2.6	<0.005	8.0 ± 1.8
Hammacher	2.8 ± 2.9	<0.005	0.1 ± 2.7
Normal (n)	3	<0.05	15
Abnormal (n)	23	<0.05	16

or variable decelerative activity in the absence of other fetal heart rate abnormality. It should also be noted that decreased variability and significant decelerative patterns were both associated with an increased incidence of acidosis in that a significant number of infants displaying these patterns were subsequently noted not to be acidotic. In this group, 50% of tracings with poor variability and 33% with severe decelerations were associated with an acidotic umbilical pH value (pH < 7.25).

In contrast, a normal fetal heart rate pattern was found to be associated with a good umbilical cord pH value and a 5-minute Apgar score considered in the normal range. Most important, no infants with normal fetal heart rate tracings were found to be acidotic and only one of the 18 had a 5-minute Apgar score of <7.

One-minute Apgar values were almost universally low if compared with those of a normal term population (85% were <7) and were found to be of no value in assessing subsequent neonatal outcome.

As noted previously, the incidence of severe intraventricular hemorrhage (grades 3 and 4),<sup>7</sup> was associated with neonatal death. However, neither neonatal respiratory distress syndrome nor intraventricular hemorrhage was found to be associated with a specific fetal heart rate abnormality.

#### Comment

Present-day results of management of the very preterm infant are encouraging with overall survival rates in the range of 70% being reported in perinatal centers with infants weighing <1001 gm.<sup>2</sup> In the present study, a 90% survival was obtained in infants with gestations of 26 to 30 weeks. The avoidance of stress associated with trauma and asphyxia and aggressive neonatal resuscitation appear to be important factors in improving perinatal morbidity and mortality.

The diagnosis of early fetal distress in the very preterm infant must therefore be of paramount importance if permanent damage or death is to be avoided. The importance of neonatal condition in subsequent outcome as evidenced by 5-minute Apgar values, umbilical cord venous pH, and base deficit changes has been reported in a recent article from this center<sup>9</sup> and

**Table VI.** Fetal heart rate tracing versus 5-minute Apgar score and pH

	5 min Apgar score	pH	p
Increased baseline (n = 8)	8.2 ± 1.2*	7.35 ± 0.05*	
Decreased variability (n = 12)	7.8 ± 1.2*	7.25 ± 0.13	<0.05
Variable decelerations (n = 4)	7.0 ± 1.9*	7.39 ± 0.06*	
Severe decelerations (n = 12)	6.5 ± 2.2*	7.28 ± 0.07 (33% < 7.25)	<0.05
Normal (n = 18)	7.8 ± 1.4 (1.8 < 7)	7.35 ± 0.05 (0% < 7.25)	

\*Not significant.

is confirmed in this study. The value of having a reliable means with which to monitor the very preterm infant in labor is therefore evident, particularly if it can be used to predict neonatal outcome. The present study would indicate that the very preterm fetus does display a higher incidence of intrapartum heart rate abnormalities as compared with that in the term fetus. When the tracing is normal, the physician can expect an uncompromised fetus during labor and anticipate a good neonatal result in the absence of other significant perinatal problems, for instance, congenital anomalies, pulmonary hypoplasia, and infections. The three infants with normal fetal heart patterns who subsequently died all had other significant perinatal factors contributing to death. No infant born after a normal tracing was subsequently demonstrated to be acidotic on umbilical cord pH measurement, and only one of the 18 received a 5-minute Apgar score of <7. Variable decelerative activity in the absence of other abnormalities was also associated with a good outcome and absence of acidosis.

These findings are a confirmation of the findings of Bowes et al.<sup>10</sup> in their study of 61 preterm infants with birth weights of <1500 gm. They are, however, at odds with the study of Westgren et al.,<sup>11</sup> which found only severe decelerative activity to be associated with acidosis. Neither Bowes' nor Westgren's study, however, found abnormal fetal heart rate patterns in the preterm infant to be associated with neonatal mortality or abnormal Apgar scores, findings distinctly different from those reported by Cibils,<sup>12,13</sup> where abnormal Apgar scores were found to be associated with abnormal fetal heart rate patterns, and from two earlier studies,<sup>14,15</sup> where abnormal patterns were found to be associated with an increase in neonatal death. The present study would indicate that, in fact, abnormal fetal heart rate patterns are associated with an increased incidence of neonatal death but are not predictive of neonatal death.

The use of quantitative scoring systems in assessing fetal heart rate patterns appears to be of value. The



two most frequently quoted are those proposed by Fischer et al.<sup>4</sup> and Hammacher.<sup>5</sup> These are both reported in the literature as being useful in the term infant, particularly in evaluating indications for fetal scalp sampling.<sup>16</sup> This paper would indicate they also have some value in assessing status of the preterm infant in labor. They certainly allow for better standardization of the reporting. Whether a better scoring system may be developed for application in the preterm infant remains to be seen.

The problem remains one of interpreting the significance of an abnormal fetal heart rate pattern just as it does in the term fetus. Some authors have recommended fetal scalp sampling as a means to evaluate fetal heart rate patterns of concern in the term fetus. Unfortunately, scalp pH values may not be an appropriate method with which to evaluate such patterns in the very preterm infant. While significant differences in cord pH values were noted between the infants who died and the control groups in this study, neonatal losses did not display the degree of acidosis typical of severe asphyxia in the term infant. Whether this represents a quantitatively different response to asphyxia in the very preterm infant such that significant problems may occur without systemic acidosis remains to be confirmed. The value of fetal scalp sampling in such a situation therefore remains in question. The report of Zanini et al.<sup>8</sup> emphasizes this problem. Forty percent of their infants with significant late decelerations were found to have a scalp pH value of  $>7.25$ . The possible complications of scalp sampling in very preterm infants also have yet to be addressed in the literature.

In summary, evidence would indicate that the very preterm infant can tolerate the stress associated with normal labor. Given a benign fetal heart rate pattern, a physician in a perinatal unit can expect both fetal well-being and a good neonatal outcome in the absence of other significant perinatal problems. The very preterm infant, however, does display an increased incidence of abnormal patterns that are associated with but not predictive of poor neonatal outcome. Additional means of evaluating the stress implied by an abnormal tracing must be developed to differentiate the preterm infant likely to have a poor outcome from that one likely to have an intact survival. To answer this question, a controlled study of scalp sampling in the very preterm fetus is presently being planned at this center. We are

looking to evaluate possible prognostic parameters other than acid-base status in assessing fetal status and subsequent neonatal outcome in the very preterm gestation.

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# Treatment of cervical intraepithelial neoplasia with electrocautery: A report of 776 cases

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From 1973 to 1984, 776 patients with cervical intraepithelial neoplasia were treated with outpatient electrocautery (hot cautery) without anesthesia. Of these, 726 (94%) were available for follow-up in 3 to 6 months. An initial cure rate with one treatment of 89% to 90% was achieved. Cure rates were similar for all degrees of dysplasia, including carcinoma in situ. There were no complications. All patients with failure of the initial treatment who returned for further outpatient management were eventually cured with use of electrocautery. Long-term follow-up rates ranged from 75% at 1 year to 46% at 5 years. There were few late recurrences, most of which were treated again (successfully) with electrocautery. Electrocautery produces cure rates similar to those for other forms of conservative management and may be the most cost-effective method of management of cervical intraepithelial neoplasia. (AM J OBSTET GYNECOL 1986;154:255-9.)

**Key words:** Cervical intraepithelial neoplasia, electrocautery, colposcopy

Conservative treatment of cervical intraepithelial neoplasia is now the accepted method of management. The advantages of conservative management as opposed to surgical removal (cone biopsy or hysterectomy) are well recognized. Methods available are electrocautery (hot cautery),<sup>1,2</sup> electrocoagulation diathermy,<sup>3</sup> cryotherapy,<sup>4</sup> and laser surgery.<sup>5</sup> All modalities report similar high cure rates. Short and long-term recurrence rates, however, are seldom reported,<sup>3,6</sup> and therefore data on the degree of permanency of effect of the different modalities of treatment is still lacking.

Electrocautery has been used for the treatment and prevention of cervical lesions, dysplastic and benign, for several decades.<sup>7,8</sup> Despite its demonstrated ability to effectively reverse dysplastic cervical lesions,<sup>1,2</sup> it has largely been discarded in favor of the newer techniques of cryotherapy and the carbon dioxide laser.

From its inception the colposcopy clinic at the Kingston General Hospital has favored and almost exclusively used electrocautery for the conservative management of cervical intraepithelial neoplasia. Results of our experience with electrocautery have been published before.<sup>2</sup> At that time, 426 patients treated with

electrocautery were described. This study is an extension of this experience, both in number of patients and length of follow-up, making this one of the largest series of the treatment of cervical intraepithelial neoplasia so far reported. This report reviews our clinic's experience with electrocautery, presenting the results of treatment in terms of initial cure rates and follow-up, and compares these results with other modalities of treatment.

## Material and methods

Since its beginning in 1973 the colposcopy clinic has seen over 2500 patients referred for assessment of abnormal cervical cytologic conditions. The general organization and policies of the clinic have been previously reported.<sup>2</sup> All patients continue to be seen by a single staff member (J. A. C.) with the resident currently serving with the oncology service. Patients are treated only after completion of a satisfactory colposcopic examination including colposcopically directed biopsies.

Electrocautery is performed in the colposcopy clinic without anesthesia. The instrument used was made by National Electric, Instrument Division, Engelhard Hanovia Inc., Long Island, New York, with a National cautery pistol tip No. 6. The entire transformation zone is treated by destroying the tissue with a series of radial burns with the red-hot metal cautery tip. Infrequently a patient with a particularly large transformation zone is treated at two sittings. This procedure differs from and should not be confused with electrocoagulation diathermy, which requires grounding of the patient

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**Table I.** Initial cure rate with treatment of cervical intraepithelial neoplasia by electrocauterization, 1973-1984

Histologic findings	Total No. treated	Total No. followed	Initial failures		Initial cure rate (%)
			No.	%	
Mild	218	203	24	11.8	88.2
Moderate	264	248	23	9.3	90.7
Marked	222	208	21	10.1	89.9
Carcinoma in situ	68	65	5	7.7	92.3
Total	772	724	73	10.1	89.9

and produces fulguration and coagulation of tissue by the passage of a high-frequency current through the tissue.

The procedure is not free of discomfort, although the expression of discomfort varies widely from patient to patient. The pain is of two types: a crampy feeling (similar to menstrual cramps) and a sensation of burning in the vagina, which usually followed too prolonged an application of the cautery tip. The latter can largely be avoided by the use of frequent intermittent applications rather than a continuous technique. The occasional very anxious patient is given an analgesic agent one-half hour before cautery (e.g., 275 mg of naproxen sodium). This may be of some value in preventing the crampy type of pain.

Treatment, once initiated, was completed in all patients. All patients were advised to expect a brownish discharge beginning 1 week after the cautery and lasting for approximately 1 week. Antibiotics were not used, nor was any local care recommended. Intercourse was discouraged until the discharge subsided. The duration of treatment, even with the most anxious patient, was usually between 3 to 5 minutes.

All patients so treated were asked to return to the colposcopy clinic for follow-up examination (cytologic study, repeat colposcopic examination, and biopsy where indicated) at 3 to 6 months. When findings of both cytologic and colposcopic examinations were negative, the patient was returned to the referring physician with the recommendation of routine follow-up, i.e., annual cytologic study.

The colposcopy clinic follows all patients treated conservatively on an annual basis. Each patient's referring physician (gynecologist or family physician) is contacted on the anniversary date of the treatment to assure that appropriate follow-up has been done and to ascertain the result of this follow-up.

## Results

From May, 1973, to September, 1984, 776 patients were treated with electrocautery for cervical intraepithelial neoplasia. Four of these patients have been ex-

cluded from analysis: In three patients results of treatment could not be adequately assessed because a hysterectomy or cone biopsy was performed by the referring physician within 2 months of treatment, and in one patient treatment with electrocautery was considered incomplete, but spontaneous regression of the dysplastic changes occurred during the follow-up period without further treatment.

Of the 772 remaining patients, 724 (94%) returned for initial follow-up assessment at 3 to 6 months. There were 73 failures of initial cautery treatment, defined as any persisting cytologic, colposcopic, or histologic evidence of dysplasia. This resulted in an initial failure rate of 10.1%, or an initial cure rate of 89.9%. Fifty-seven patients had minor cytologic abnormalities on the first follow-up visit, which reverted to normal within 3 to 6 months without further treatment (transient mild dysplasia). These patients were not considered as treatment failures. Cure rates were similar for all degrees of dysplasia including carcinoma in situ, as is illustrated in Table I.

Table II shows the outcome of the patients who had failure of the initial electrocautery treatment. Most of the patients who had persisting dysplastic changes following the initial cautery were retreated a second, or occasionally third, time with electrocautery. After the initial cautery one patient had persisting cytologic abnormalities associated with condyloma of the cervix and was eventually cured with podophyllum resin applications. Another patient has been retreated with electrocautery in another center. The remainder of the patients with failure of initial cautery and not treated with repeat cautery were either treated with cone biopsy (4 patients) or hysterectomy (4), were lost to follow-up (3), or are currently awaiting further evaluation and/or treatment with electrocautery (2).

There were two patients who did not return to our clinic for their follow-up assessment, but were subsequently treated elsewhere with cryotherapy because of mild cytologic abnormalities on examination 8 to 9 months after our treatment with electrocautery. The original histologic diagnosis in these patients had been of moderate and marked dysplasia. If these patients are included in the treatment failures, then the initial failure rate would be 10.3% (75 of 726).

There were four patients who had recurrences diagnosed between 7 and 12 months after treatment, following an initial negative assessment at 3 to 6 months. If these patients are considered to have persistent disease rather than recurrences, then the initial failure rate increases to 79/726, or 10.9%. All four of these patients were retreated successfully with electrocautery.

There was only one occurrence of microinvasive cancer following treatment with electrocautery. The patient was treated with electrocautery with a histologic diagnosis of moderate dysplasia but did not return for



**Table II.** Results of re-treatment

<i>Histologic findings</i>	<i>No. of failures</i>	<i>No. having second treatment</i>	<i>No. followed up after re-treatment</i>	<i>No. cured</i>	<i>No. having third treatment</i>	<i>No. cured</i>
Mild	24	18	16	16	—	—
Moderate	23	17	15	13	1	1
Marked	21	19	16	11	3	3
Carcinoma in situ	5	4	4	4	—	—
Total	73	58	51	44	4	4

follow-up until 8 months later, at which time diagnosis of persistent disease was made. She did not return again to the clinic, but after an additional 5 months a vaginal hysterectomy was performed because of uterine prolapse. Histologic examination of the cervix revealed dysplasia and carcinoma in situ as well as one small discrete focus of microinvasion of <1.0 mm in the depth of an endocervical gland. We are unaware of any other occurrence of invasive cancer following our treatment with electrocautery.

Of the seven patients who experienced failure of the second treatment with electrocautery, four underwent a third treatment, and three patients had a cone biopsy. All four patients who had a third treatment were cured, having normal cytologic and colposcopic findings on follow-up. Thus all patients who continued to return to the colposcopy clinic for outpatient management were eventually cured with use of electrocautery.

Favorable long-term follow-up rates have been achieved. Table III shows the number of patients who were followed at various time intervals. Follow-up rates were similar for all degrees of dysplasia, although there was a consistently higher follow-up rate of patients with carcinoma in situ (Table IV).

Recurrences were defined as any cytologic, colposcopic, or histologic evidence of dysplasia (other than a transient mild cytologic abnormality) occurring more than 6 months after cautery. Among the 463 patients followed for 1 year, there were four recurrences (0.9%), occurring between 7 and 12 months. These might be considered as failures of primary treatment rather than recurrences, as previously mentioned. There were three recurrences in 267 patients at 2 years (1.1%) and four recurrences in 148 patients at 3 years (2.7%). There have been no late recurrences after 3 years, although there has now been follow-up of some patients for more than 8 years after treatment. All but two of the patients with recurrences had both negative cytologic and negative colposcopic examinations following their initial treatment. The other two patients did not return to the clinic until the time of their recurrence, although they both had negative cytologic examinations, which were performed elsewhere in the intervening time interval. Table V outlines details regarding the patients with recurrences.

Eight of the 11 patients with recurrences more than

**Table III.** Length and percentage of follow-up

<i>Length of follow-up (mo)</i>	<i>No. treated</i>	<i>No. followed up</i>	<i>Percent followed up</i>
3-6	772	726	94.0
12	622	465	74.8
24	415	268	64.6
36	265	148	55.8
48	173	90	52.0
>60	109	50	45.9

6 months after treatment returned to the colposcopy clinic for management and were treated successfully with repeat electrocautery. One patient was treated by hysterectomy; one patient has been re-treated but has not yet returned for follow-up, and the remaining patient has not yet returned to the colposcopy clinic for management.

Treatment with electrocautery was free of significant complications. Several patients reported a persistent mild bleeding for up to 2 to 3 weeks after surgery. This bleeding always eventually ceased, and from our early experience it was found unrewarding to try to deal with this problem by repeat cautery of the bleeding areas. No patient required hospitalization, blood transfusion, or sutures to the cervix. The complication of cervical stenosis was not seen although two or three patients had synœchia between anterior and posterior lips of the cervix, which were either broken down or disregarded.

### Comment

This study demonstrates a cure rate of 89% to 90% with a single treatment of electrocauterization (hot cautery) in the treatment of cervical intraepithelial neoplasia. All patients with failure of the initial treatment who continued in the program of outpatient management with electrocautery were cured with a second or rarely a third treatment. This compares favorably with results obtained with the other modalities of conservative treatment.<sup>3-5, 9-14</sup>

There was no difference in the success of treatment with electrocautery for the different degrees of dysplasia, including carcinoma in situ. This is in contrast to results for cryotherapy, where some series have found a considerably higher failure rate in the treatment of marked dysplasia and carcinoma in situ.<sup>4, 9, 10</sup>

**Table IV.** Follow-up by severity of disease

Severity of disease	Patients followed up at each time interval											
	3-6 months		1 year		2 years		3 years		4 years		5 years	
	n	%	n	%	n	%	n	%	n	%	n	%
Mild	203/218	93	129/171	75	65/105	62	36/70	51	23/46	50	10/24	42
Moderate	249/264	94	167/221	76	103/157	66	59/99	60	33/64	52	20/43	47
Marked	209/222	94	126/176	72	77/119	65	35/70	50	17/39	44	11/25	44
Carcinoma in situ	65/68	96	43/54	80	23/34	68	18/26	69	17/24	71	9/17	53

**Table V.** Recurrences and outcome

Case No.	Time from first treatment to diagnosis of recurrence	Severity of diseases		Re-treatment with cautery	Result of re-treatment	Comment
		Initial	Recurrence			
1616	7 mo	Mild	Mild	Yes	Cure	Cytologic study done elsewhere negative at 3 months; first follow-up visit in clinic at 7 months
1112	7 mo	Moderate	Mild	Yes	Cure	
1374	8 mo	Moderate	Moderate	Yes	Cure	Mild dysplasia on cytologic study but biopsy negative
1660	11 mo	Marked	Mild	Yes	Cure	
238	2 yr	Mild	Mild	Yes	Cure	Mild and moderate dysplasia on cytologic study, but biopsy negative; re-treatment; follow-up negative
1308	2 yr	Moderate	Moderate	Yes	Cure	
1646	2 yr	Moderate	Marked	Yes	Not known	Cytologic study done elsewhere negative at 11 and 21 months; first follow-up in clinic at 2 yr showed marked dysplasia; re-treatment November, 1984; patient has not returned for follow-up
1201	3 yr	Mild	Mild	No	—	Recurrence diagnosed elsewhere on cytologic study; patient has not returned to clinic for evaluation
546	3 yr	Moderate	Mild	Yes	Cure	Mild dysplasia on cytologic study but biopsy negative Normal examination in clinic at 1 year; subsequent cytologic study (done elsewhere) showed moderate dysplasia at 3 years and marked and carcinoma in situ at 5 years; hysterectomy at 5 years; carcinoma in situ
846	3 yr	Moderate	Mild	Yes	Cure	
404	3 yr	Marked	Moderate	No	—	

**Table VI.** Disposition of all new patients, January 1, 1984, to June 30, 1984

Diagnosis and treatment	No.	%
Electrocautery for cervical intraepithelial neoplasia	100	49.3
Cone biopsy for inadequate colposcopic examination	6	3.0
Cervical intraepithelial neoplasia present; returned to referring gynecologist (in another center); treatment by electrocautery recommended	9	4.4
Hysterectomy (for other benign disease)	2	1.0
Cervical intraepithelial neoplasia in pregnancy; did not return after delivery	5	2.5
Refused colposcopic examination, biopsy, or treatment	5	2.5
Invasive cancer of the cervix	4	2.0
Vaginal intraepithelial neoplasia; treated with laser	2	1.0
Vulvar intraepithelial neoplasia; laser treatment or vulvectomy	4	2.0
Electrocautery for benign cervical disease (e.g., condyloma without dysplasia, cervicitis)	25	12.3
No evidence of dysplasia; no treatment required	41	20.2
Total	203	100

although this has been refuted by other studies.<sup>11, 12</sup> Interestingly, most of our recurrences had an initial diagnosis of mild or moderate dysplasia. Only two of the 11 patients with recurrences more than 6 months

after treatment had an initial diagnosis of marked dysplasia, and there have been no recurrences in those patients treated for carcinoma in situ.

In the first 2 to 3 years, only 3% of the first 200

patients seen in the clinic were treated with electrocautery, since the clinic served primarily as a diagnostic center, with patients being sent back to the referring physician for treatment. In 1976 and 1977 conservative treatment (by electrocautery) was performed in the clinic more often, with 16% of the next 300 patients being so treated, although conservative treatment was still being reserved for those patients desiring to preserve their fertility. Since then, as confidence grew in the ability to cure these lesions with electrocautery, *all* patients who were appropriate candidates for conservative management were recommended for treatment with electrocautery. Currently, 45% to 55% of patients referred are eventually treated with outpatient electrocautery in the clinic. Most of the remaining patients seen in the clinic are found not to have cervical intraepithelial neoplasia. The eventual disposition of all new patients seen in the clinic in the first 6 months of 1984 is listed in Table VI and demonstrates the almost universal use of electrocautery for dysplasia and carcinoma in situ of the cervix.

Acquiring good long-term follow-up is difficult because the patient population tends to be young and itinerant; but it is important, since the natural history of cervical intraepithelial dysplasia can extend over several years.<sup>1, 15</sup> Our long-term follow-up shows that late recurrences, while uncommon, may still occur several years after successful treatment, confirming this need for long-term follow-up. However, in most patients electrocautery appears able to effect a permanent reversal of the premalignant changes.

Reports on the other modalities of conservative management indicate that a small percentage of patients have complications that we have not seen from treatment with electrocautery. Significant bleeding complications have been reported in from 0%<sup>15</sup> to 2.3%<sup>16</sup> to 5.6%<sup>3</sup> of patients treated with laser vaporization, and complications of hemorrhage, infection, and cervical stenosis have been reported in up to 3% treated with electrocoagulation diathermy.<sup>3</sup> Complications of significant bleeding or pelvic infection are occasionally reported following cryotherapy.<sup>6, 11</sup> General or regional anesthesia is not required for electrocautery, although it usually is for adequate treatment with electrocoagulation diathermy<sup>3</sup> and for some patients undergoing laser vaporization procedures.<sup>5</sup>

Recently a review comparing cryotherapy and the carbon dioxide laser for the treatment of cervical intraepithelial neoplasia found that, while the two methods had similar effectiveness, cryotherapy was considerably less expensive in both capital and maintenance costs and it was technically easier to perform.<sup>14</sup> The equipment required for electrocautery is less expensive

than either of these modalities. The cost of a machine for electrocautery is currently approximately \$500 (U.S.), and maintenance costs are almost nonexistent. Special training in the use of the equipment is not required, as it is for laser surgery,<sup>16</sup> and the time required in treating with electrocautery is less than is generally reported for either laser surgery<sup>10</sup> or cryotherapy.<sup>11</sup>

Electrocautery (hot cautery) is as effective in treating cervical intraepithelial neoplasia as the other methods available for conservative management. In addition to the advantages of lack of need for anesthesia, fewer significant complications, and ease of use, it is also the most cost-effective method of treatment available.

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# Cervical intraepithelial neoplasia and condyloma: An analysis of diagnostic accuracy of posttreatment follow-up methods

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The appropriate follow-up method of patients treated for cervical intraepithelial neoplasia and condyloma is controversial. One school of thought favors cytologic testing alone, whereas the other favors cytologic testing combined with colposcopy. We have analyzed the records of 750 patients treated for cervical intraepithelial neoplasia or condyloma or both in our Colposcopy Clinic. Cytologic testing, colposcopy, and histologic examination were done routinely at 3 to 4, 6 to 8, and 12 to 15 months after treatment of the above conditions in 750 patients. Totals of 95% and 5% of all 128 persistent lesions were detected by histologic examination at the first (3 to 4 months) and second (6 to 8 months) posttreatment visits, respectively. The combined false negative rates for both visits were 23% for cytologic testing, 8% for colposcopy, and 5% for histologic examination. False negative rates at first visit were 1.5% for colposcopy combined with cytologic testing. These observations suggest that colposcopy and, if appropriate, histologic examination significantly contribute to cytologic testing for diagnosing the majority of persistent disease during the first posttreatment visit. Cytologic testing alone seems to be sufficient for the subsequent follow-up of patients treated for cervical intraepithelial neoplasia and/or condyloma. (AM J OBSTET GYNECOL 1986;154:260-4.)

**Key words:** Cytology, colposcopy, cervical intraepithelial neoplasia, follow-up

Better understanding of the natural history of cervical cancer precursors in the past decade led to the outpatient management of most women with cervical intraepithelial neoplasia.<sup>1</sup> Rigid diagnostic criteria including appropriate cytologic testing, colposcopy, endocervical curettage, and multiple punch biopsies have been recommended to obtain good therapeutic results, on the one hand, and to make sure that invasive carcinoma is not missed, on the other.<sup>1,2</sup> The appropriate means to follow up women treated for cervical cancer precursors on an outpatient basis have not been as rigidly established, and in fact the issue remains controversial. Two basic schools of thought exist with regard to methods used for posttreatment follow-up. One recommends the patient to be evaluated by cytologic testing alone,<sup>1,3-5</sup> whereas the other favors the combined use of cytologic testing and colposcopy at regular intervals at least during the first year after outpatient treatment.<sup>6-8</sup>

The purpose of this study was to evaluate each diagnostic technique's relative sensitivity for detecting residual disease independently of each other in the same patient. It was hoped that such evaluation would provide data on the most effective approach for evaluating patients during the first year after the last treatment received.

## Material and methods

The charts of 750 patients who were treated for cervical intraepithelial neoplasia with (35%) or without (25%) koilocytotic features and cervical condyloma (40%) in the Colposcopy Clinic of The Sir Mortimer B. Davis Jewish General Hospital were analyzed. In all these cases, cytologic testing, colposcopy, and histologic examination (endocervical curettage and biopsy) were done prior to treatment. Cellular and histologic sampling as well as colposcopic examination was performed alternately by residents in obstetrics and gynecology and gynecologists in training in colposcopy as well as by the senior author (A. F.). In all cases, the lesional margins were visualized and the endocervical canal was free of disease on colposcopy and negative endocervical curettage. The treatment modalities of the patients included cryotherapy (60% of the cases), laser vaporization (35%), excisional biopsy (2.5%), and 5% 5-fluorouracil (Efudex) cream (2.5%). Follow-up appointments for repeat cytologic testing, colposcopy, endocervical curettage, and/or biopsy were performed at 3 to 4, 6 to 8, and 12 to 15 months after treatment. If all

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**Table I.** False negative rates of cytologic testing and colposcopy at 3 to 4 and 6 to 8 months after treatment

	<i>Follow-up visit</i>					
	<i>First (3-4 mo)</i>		<i>Second (6-8 mo)</i>		<i>Total (3-8 mo)</i>	
	<i>No.</i>	<i>%</i>	<i>No.</i>	<i>%</i>	<i>No.</i>	<i>%</i>
Residual disease found by histologic examination:	122	95	6	5	128	100
False negative						
Cytologic testing	23	19	1	17	24	19
Colposcopy	4	3	0	0	4	3

\* $p < 0.05$ .

these investigations were negative, the patient was discharged and followed up by her own private physician. If residual disease was found, the patient was treated according to localization and size of the lesional tissue.

Cytologic testing was performed by os aspiration followed by circumferential scraping of the exposed portion of the cervix with the use of Acupap.\* The specimen was evenly spread on a glass slide and fixed immediately. Colposcopic examination was performed after application of 5% fresh acetic acid solution over the exposed portion of the cervix uteri. Evaluation of the cervical epithelium was based on well-known colposcopic patterns previously described by Kolsad and Staff.<sup>9</sup> Curettage of the endocervical canal was performed by the Kevorkian curette starting from the isthmus down to the external os, circumferentially twice. The material obtained was placed on a lens paper and fixed immediately in Bouin's solution. Biopsy specimens were taken by Kevorkian biopsy forceps, and the specimen was placed on a piece of lens paper with the mucosal surface upward. The specimen was immediately placed in Bouin's fixative upside down.

The cytology smears were read by cytotechnicians in the usual manner, with knowledge of the source (Colposcopy Clinic) of the specimens. All pathologic specimens were read by one of the authors (A. F.). Cytologic criteria of cervical intraepithelial neoplasia and condyloma were presence of malignant basal and parabasal cells and koilocytes and dyskeratocytes, respectively. Histologically, cervical intraepithelial neoplasia was defined as an abnormal epithelium in which disturbed cellular organization and maturation were associated with nuclear aneuploidy and abnormal mitotic figures.<sup>10</sup> When koilocytes occupied the upper one half to one third of the epithelium, the diagnosis of cervical intraepithelial neoplasia with koilocytotic features was given. Ordinary condylomatous lesions (condyloma planum) contained koilocytotic, squamous superficial or intermediate cells with prominent, irregular, glycogen-poor cytoplasmic cavities and degenerated nuclei

and often dyskeratotic cells engaged in individual cell keratinization. By definition, ordinary condylomas were devoid of cells with nuclear aneuploidy and abnormal mitotic figures except for tripolar mitosis and a dispersed metaphase.<sup>10</sup> All cases in which the smears were originally interpreted as negative but colposcopy and histologic examination showed abnormalities were reviewed by one of the authors (A. F.). The statistical significance of the differences in diagnostic yield between cytologic testing, colposcopy, and histologic examination were analyzed by Student's *t* test. When  $p$  was  $< 0.05$ , the differences were considered statistically significant.

### Results

Overall, 128 patients were found by histologic examination to have residual (persistent) lesions. One hundred twenty-two (95%) of these were detected at the first posttreatment visit at 3 to 4 months and 6 (6%) at the second (6-8 months) visit. No patients were found to have residual disease at 12 to 15 months after initial treatment (Table I). Totals of 3% and 19% of the 122 patients with disease diagnosed by histologic examination were not detected by colposcopy and cytologic testing, respectively (Table I). In two of the four cases (50%) in which results of colposcopy were negative (all located in the external os), but results of histologic examination were positive, results of cytologic testing were also positive. The combined false negative rates of colposcopy and cytologic testing at first visit were two of 122 patients or 1.5%. Results of colposcopy were positive, confirmed by histologic examination in 20 of 24 (83%) cases with cytologically negative results. All six cases that were diagnosed at the second posttreatment visit (6 to 8 months) were missed by cytologic testing, colposcopy, and histologic examination at the first posttreatment visit (3 to 4 months). Assuming that all six of these lesions were present at the first visit, the combined false negative rates of all diagnostic means for both visits were 23% for cytologic testing, 8% for colposcopy, and 5% for histologic examination (Table II). Cytologic testing done at the second visit failed to

\*Unimar.

**Table II.** Combined false negative rates for both visits according to diagnostic means

Diagnostic means	False negative results at first and second visits (n = 128)	
	No.	%
Cytologic testing	30	23
Colposcopy	10	8
Histologic examination	6	5

\*Includes the six cases that were missed by all diagnostic means at first follow-up visit.

detect one of the six cases (17%) diagnosed by colposcopy and histologic examination (Table I). The pretreatment and posttreatment diagnoses in the 30 cases that were missed by cytologic testing were similar and consisted of three cases of cervical intraepithelial neoplasia, 13 of cervical intraepithelial neoplasia with koilocytotic features, and 14 cases of condyloma planum. To determine the proportion of true false negative smears and those due to laboratory reading errors, all smears that were originally read as negative in the face of positive colposcopic and histologic examination were reviewed by A. F. The review showed that 80% of the smears contained no cytologic abnormalities (true false negative), whereas 20% of the smears were underread (reading error).

Posttreatment residual lesions that were missed by cytologic testing were located peripherally on the exocervix alone (21%), in the external os alone (54%), or in both the external os and exocervix (25%). There was no correlation between the location of persistent disease and treatment modality. Approximately the same number of patients received laser and cryotherapy in the residual disease group. Table III contains the respective locations of persistent disease as related to type of treatment received. In the six cases that were missed at first posttreatment visit by all diagnostic means, lesions were located at the external os at the second visit. They were all relatively small (<5 mm) and had a focal distribution within the os. They were detected by colposcopy and confirmed by endocervical curettage. Endocervical curettages were not obtained in these cases at the first posttreatment visit because there was no colposcopic evidence of lesional tissue in the external os.

#### Comment

In the present study, the sensitivities of cytologic testing, colposcopy, and histologic examination in diagnosing residual or persistent disease after outpatient therapy for cervical intraepithelial neoplasia and/or condyloma (treatment failures) were analyzed and compared with each other. According to the results, the

**Table III.** Correlation between location of residual disease and type of treatment in the 24 cases with false negative cytologic testing

Location of residual disease	Type of treatment	
	Laser therapy (n)	Cryotherapy (n)
Exocervix*	3	2
External os	6	7
Both	3	3
Total cases	12	12

\*Peripheral location on portio.

vast majority of persistent disease (95%) was detected at the first posttreatment visit at 3 to 4 months and the remainder (5%) at the second follow-up visit at 6 to 8 months. All other patients remained disease-free for at least 1 year after treatment. While none of the diagnostic means had 100% sensitivity in detecting posttreatment residual disease, the false negative rates of cytologic testing were comparatively higher (23%) than those of colposcopy (8%) and histologic examination (5%). Moreover, false negative results of colposcopic and histologic examination occurred only at the first visit, whereas the false negative rate of cytologic testing at the second visit was similar (17%) to that observed at the first posttreatment visit (19%).

False negative rates for cytologic testing have been estimated to range between 5% and 50%.<sup>8, 11, 12</sup> There are several causes of false negative cytologic results.<sup>1, 11, 12</sup> These include poor technique in taking and preparing smears, lack of exfoliation of abnormal cells at the time of cytologic sampling, and failure by the cytologist to recognize malignant cells on the smears. In the first two instances, the false negative cytologic result is referred to as true false negative, for even on thorough review of the smears no abnormal cells can be found, yet colposcopically and histologically disease is present on or in the cervix. When review of the smear that was read as negative reveals presence of abnormal cells, then it is considered to be a laboratory error false negative. The proportion of true false negative versus laboratory error false negative smears ranges between 80% and 20% in some series and between 22% and 78% in others.<sup>13</sup> The precise reasons for the diametrically opposed reports are not clear but different techniques used for taking Papanicolaou smears and the reading quality of laboratory personnel probably represent important factors. In our present experience, the true false negative-laboratory error false negative smear ratio was largely in favor of the former, that is, 80% versus 20%, thus confirming the experience of North American investigators.<sup>11, 12</sup> The relatively high false negative rates (23%) and particularly the true false



negative rates in the present study are surprising for we used the "combination" technique for obtaining cervical cytologic smears. It has been suggested previously that true false negative rates may be considerably reduced (5% to 10%) when scraping of the squamocolumnar junction of the transformation zone is combined with aspiration of the external os.<sup>1,2</sup> There may be several explanations for the discrepancies in the results. The studies were carried out on chiefly untreated cervixes where most lesional areas were presumably more extensive and thus relatively easy to reach. In our study, the vast majority of residual lesions had a diameter of <1 cm and were located either in the external os or at the periphery of the portio where cryotherapy or laser therapy apparently failed to destroy the external margins of the lesional mucous membranes. Small-sized lesions and those with unusual locations on or in the cervix are evidently more difficult to reach than large, well-established lesions, despite use of the combination technique. Even histologic examination failed to detect a few small lesions hidden within the external os in this series. Unlike in previous studies, which were carried out under strict experimental conditions,<sup>14</sup> in our study the Papanicolaou smears were taken by obstetrics and gynecology residents and gynecologists trained in colposcopy with different technical skills and experience without knowledge of the study. The purpose of this approach was to create a situation closer to that of routine practice. Another factor that may be partly responsible for the relatively high false negative rates of cytologic testing in our study might be the different sampling device used. In the study of Richart and Valiant,<sup>14</sup> plastic cytopipettes and the Ayre wooden spatulas were used, whereas we used a plastic device which served as both an aspirator and a scraper (Acupap). Whether cell adhesion was less adequate on the plastic surface of the Acupap than on the wooden spatula is not known. The surface of the Acupap sampler has fine serrations that serve to entrap exfoliated squamous cervical cells and thus it seems comparable in design to the ragged surface of the Ayre spatula.

Histologic examination detected the vast majority of residual lesions (95%) at the first posttreatment visit at 3 to 4 months. Cytologic testing, however, failed to detect a relatively significant number of cases of posttreatment residual disease at the first as well as at the second visit. The data suggest that the maximum diagnostic sensitivity of posttreatment cytologic testing is about 80%, mainly because of the location and small size of persistent lesions. Similar results were reported by others.<sup>3-7</sup> While colposcopy had comparatively low false negative rates, these can be further decreased by cytologic testing. Indeed, two of the four cases missed by colposcopic examination at the first visit were detected by cytologic testing. As a result, cytologic testing

is complementary to colposcopy with a 1.5% combined false negative rate at the first posttreatment visit. Similarly colposcopy had lower false negative rates than histologic examination. In fact, colposcopy missed only four histologically verified lesions at the first follow-up visit. These were located deep in the external os with no evidence of disease on the exocervix and were detected by endocervical curettage. The other six cases missed by colposcopy were also missed by histologic examination but detected by colposcopy and endocervical curettages at the second posttreatment visit at 6 to 8 months. Histologic sampling of the external os was performed because of a colposcopic impression of lesional tissue in the os. Such impression was not recorded at the time of the first posttreatment visit. In view of the high diagnostic sensitivity of colposcopy, histologic sampling of the cervix is not recommended at the first posttreatment visit at 3 to 4 months unless colposcopy is suggestive of or consistent with presence of residual disease.

In conclusion, it appears from this study that the best approach to detect the largest number of treatment failures within the shortest period of time is by combined cytologic testing and colposcopy, and if appropriate, histologic examination at the first posttreatment visit at 3 to 4 months. Curettage of the endocervical canal and the external os is particularly useful to reveal lesional tissue with a deep external os location. On the other hand, at the second follow-up visit at 6 to 8 months, the number of residual cases is too small (5%) to justify routine colposcopy on a cost-effective basis. Cytologic sampling with the combination technique (external os aspiration and scraping of the exocervix) at 6 to 8 months after treatment and at all subsequent visits appears adequate for a safe and sound follow-up protocol of patients treated on an outpatient basis for cervical intraepithelial neoplasia and/or condyloma. The question as to whether the same follow-up protocol should be applied to patients who were treated surgically for cervical intraepithelial neoplasia on an inpatient basis cannot accurately be answered from this study. However, failure rates and recurrences after conization are similar to those observed after cryotherapy and laser therapy.<sup>15</sup> As a result, these patients may also benefit from a thorough cytologic-colposcopic examination at 3 to 4 months after conization. Posthysterectomy failures and recurrences are low enough to justify follow-up examinations with the use of cytologic testing alone.<sup>16</sup>

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## Cervical carcinoma in women aged 34 and younger

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This presentation addresses three questions concerning invasive cervical carcinoma in women 34 years of age and younger. Is there an increase in the incidence of the disease? Is it more or less susceptible to prevention by cervical screening? Is the clinical behavior different for this age group? Three separate studies are reported: (1) Incidence data in the younger age group have been reviewed at the national, provincial, and local levels. (2) Cytologic screening histories of 125 patients who subsequently developed cervical carcinoma were reviewed. (3) The clinical histories of 121 women 34 years of age and younger, with invasive cervical carcinoma, were reviewed and compared with those of 242 control women 35 years of age and older. Results indicate an increase in incidence in the younger age group in the three prairie provinces only. Cytologic histories are similar except for an increase in false negative reports in the younger age group. Clinical behavior of the disease is similar for both age groups. (*AM J OBSTET GYNECOL* 1986;154:264-9.)

**Key words:** Cervical carcinoma, screening, cytologic results

There has been a general impression that the incidence of invasive cervical carcinoma in women 34 years of age and younger is increasing.<sup>1-3</sup> Other observations in the literature suggest that younger women might be less susceptible to prevention of invasive cervical carcinoma by cervical screening.<sup>4,5</sup> There is also a devel-

oping concern that the preinvasive disease is more aggressive and reaches the invasive stage more quickly in the younger age group.<sup>6,7</sup> This presentation attempts to answer these questions by reviewing three separate patient groups: Group 1, incidence data at the national, provincial, and local levels of the invasive disease in women 34 years of age and younger; Group 2, cytologic data consisting of screening histories of women 34 years of age and younger as compared with those of women 35 years of age and older, all of whom subsequently developed invasive cervical carcinoma; Group 3, clinical data consisting of the clinical patterns of the disease in younger and older women treated at the Ontario Cancer Treatment and Research Foundation, Kingston clinic.

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Fig. 1. Proportion of women 34 years old and younger, diagnosed as having invasive cervical carcinoma.

## Material and methods

### Group 1

**Incidence data.** These data include the national and provincial age-adjusted incidence rates of invasive cervical carcinoma obtained for the years 1971, 1975, and 1980 from the Ontario Cancer Treatment and Research Foundation, Toronto. Incidence data were also obtained from two local centers (Princess Margaret Hospital, Toronto, and the Ontario Cancer Treatment and Research Foundation, Kingston clinic).

### Group 2

**Cytologic data.** These data were based on the cytologic histories of 125 consecutive patients with invasive cervical carcinoma registered at the Ontario Cancer Treatment and Research Foundation, Kingston clinic, between January, 1973, and December, 1984. Thirty-five of these patients were 34 years of age and younger and the remaining 90 patients were 35 years of age and older. At the time of diagnosis both younger and older patients underwent a detailed interview of past cytologic screening. The patients' prior physicians were contacted requesting information on the patient's past cytologic screening, dates of examinations, cytologic reports, and laboratories used. When available, previous cytologic slides were reviewed by our cytopathologist. Information sought from this review included the frequency of undercalled and overcalled cytologic results, differences in response by physicians and/or patients to abnormal cytologic results, and the number of negative cytologic smears obtained prior to the onset of invasive disease.

### Group 3

**Clinical data.** From 1950 to 1984 inclusive, 121 women 34 years of age and younger were treated for invasive cervical carcinoma at the Ontario Cancer Treatment and Research Foundation, Kingston clinic. Their histories beginning at the time of diagnosis of

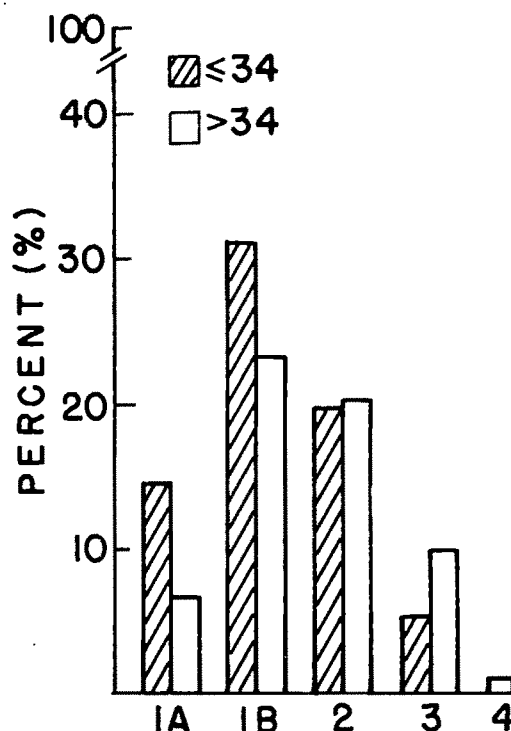


Fig. 2. The stage (International Federation of Gynecology and Obstetrics) distribution of invasive cervical carcinoma among women  $\leq 34$  or  $> 34$  years of age who had had at least one negative cytologic smear within 3 years before the diagnosis.

the invasive disease were reviewed. A control group of 242 patients 35 years of age and older with invasive cervical carcinoma treated during the same time period were similarly reviewed. For each case there were two controls, the patient with invasive cervical carcinoma, 35 years of age and older, registered immediately before and immediately after the 34-year-old and younger patient. The review of the history included cell type, degree of differentiation, stage, method of treatment, response to treatment, and survival. Accuracy in data collection was assessed after each group of 10 charts when the authors exchanged charts and data recording sheets with each other for review.

Univariate analysis comparing younger and older women on all cytologic and clinical data collected was made with the use of  $\chi^2$  analysis and  $t$  tests. The survival of younger and older women was assessed by the life-table method of analysis.

## Results

### Group 1

**Incidence data.** The age-adjusted incidence rates of invasive cervical carcinoma for women 34 years of age and younger indicate little change at the national level during the last decade. The age-adjusted incidence rate changed from 4.01 cases per 100,000 in 1971 to 3.71



**Table I.** Age-adjusted incidence rate of cervical carcinoma in women 34 years of age and younger (per 100,000 population) in Canada and the provinces, 1971, 1975, and 1980\*

Province	1971		1975		1980	
	Mean	SE	Mean	SE	Mean	SE
Canada†	3.10	0.28	3.24	0.26	3.97	0.27
Canada	4.01	0.25	3.39	0.21	3.71	0.21
Newfoundland	5.17	1.95	6.82	2.01	2.37	1.07
Prince Edward Island	—	—	—	—	2.39	2.41
Nova Scotia	3.39	1.28	2.33	0.96	3.81	1.16
New Brunswick	5.01	1.77	4.35	1.46	3.96	1.27
Quebec	2.60	0.37	1.19	0.24	3.39	0.39
Ontario	5.65	0.50	3.77	0.37	3.10	0.31
Manitoba	3.03	1.07	7.28	1.53	8.94	1.59
Saskatchewan	1.35	0.78	3.21	1.14	4.90	1.28
Alberta	2.49	0.72	2.82	0.69	3.09	0.61
British Columbia	4.89	0.88	6.61	0.78	4.22	0.67

Special thanks to Dr. E. A. Clarke and Ms. R. Dolinsky of the Ontario Cancer Treatment and Research Foundation, Toronto, for preparation of the age-adjusted incidence rates.

\*Rates are age-adjusted with the world population used as a standard.

†Canada does not include Ontario, the Yukon, or Northwest Territories.

**Table II.** Additional cytologic information addressed in the analysis of the patients' past cytologic screening history

Variable	Younger group (35 patients*)		Older group (90 patients)	
	No.	%	No.	%
Women appropriately screened†	27	79.4	64	71.1
Women with one or more undercalled cytologic smear‡	20	58.8§	33	36.7
Women with one or more overcalled cytologic smear‡	31	91.2	86	95.6

\*Missing data from one patient.

†Two or more smears within 5 years, three or more smears within 10 years, excluding smears within 3 months of the patient's anniversary date.

‡According to the classification mild, moderate, and severe dysplasia and carcinoma in situ a slide was considered undercalled when the original diagnosis was at least two levels less than that of the reviewing cytopathologist. A slide was considered overcalled when the original diagnosis was at least two levels greater than that of the reviewing cytopathologist.

§ $\chi^2$  Analysis = 4.09 with 1 df,  $p < 0.05$ .

|| $\chi^2$  Analysis = 0.26 with 1 df,  $p < 0.61$ .

cases per 100,000 in 1980 (Clarke AE, personal communication). However, at the provincial level increases in age-adjusted incidence rates have taken place in the three prairie provinces (Manitoba, Saskatchewan, and Alberta, Table I). In Ontario, the age-adjusted incidence rate indicated a decrease during the last decade.

Incidence data collected from two local centers (Princess Margaret Hospital, Toronto, and the Ontario Cancer Treatment and Research Foundation, Kingston clinic) indicate that, of all invasive cervical carcinoma cases reported since 1960, there has been an increase in the proportion of women 34 years of age and younger (Fig. 1). However, from 1975, this increase was more apparent, from 6.8% in 1975 up to 13.6% in 1983.

#### Group 2

*Cytologic data.* All 125 women whose cervical cytologic

history was reviewed, between 1973 and 1984, had at least one negative cytologic smear within 10 years of the diagnosis of the invasive disease. Twenty-five (71.4%) younger women and 55 (61.1%) older women had at least one negative cytologic smear within 3 years preceding the diagnosis of the invasive disease. This difference was not significant ( $\chi^2$  analysis = 0.8 with 1 df,  $p = 0.38$ ).

The relationship between stage of invasive disease and at least one negative cytologic smear within 3 years before the diagnosis of invasive disease, for younger and older women, is presented in Fig. 2. Younger women having at least one negative cytologic smear within 3 years preceding the diagnosis of invasive disease presented with a less advanced stage as compared with that in older women but this relationship was not significant ( $\chi^2$  analysis = 4.46 with 3 df,  $p = 0.22$ ).

**Table III.** The distribution of stage of invasive cervical carcinoma for younger and older women at the time of diagnosis

Stage*	Younger women		Older women	
	No.	%	No.	%
IA	13	10.7	12	5.0
IB	65	53.7	92	38.0
II	22	18.2	72	29.8
III	16	13.2	48	19.8
IV	2	1.7	15	6.2
Not staged	3	2.5	3	1.2

\* $\chi^2$  Analysis = 19.0 with 5 df,  $p < 0.002$ .

**Table IV.** The distribution of cell type of invasive cervical carcinoma for younger and older women at the time of diagnosis

Cell type*	Younger women		Older women	
	No.	%	No.	%
Squamous cell carcinoma	111	91.7	218	90.1
Adenocarcinoma	9	7.4	22	9.1
Adenocarcinoma-squamous cell carcinoma	1	0.8	1	0.4
Undifferentiated small cell carcinoma	0		1	0.4
Undifferentiated large cell carcinoma	0		0	

\* $\chi^2$  Analysis = 1.03 with 3 df,  $p = 0.8$ .

**Table V.** The distribution of degree of differentiation of invasive cervical carcinoma for cases and controls at the time of diagnosis

Degree of differentiation*	Younger women		Older women	
	No.	%	No.	%
Well differentiated	17	14.0	22	9.1
Moderately differentiated	22	18.2	53	21.9
Poorly differentiated	23	23.1	50	20.7
Not stated	5	44.6	117	48.3

\* $\chi^2$  Analysis = 2.86 with 3 df,  $p = 0.41$ .

Several additional questions regarding cytologic history in younger and older women were analyzed. Table II indicates the three main additional questions addressed by the cytologic data. The results indicate no significant differences between the two age groups in their cytologic history except that in younger women, compared with older women, the cytologic test results were significantly more likely to be undercalled ( $\chi^2$  analysis = 4.09 with 1 df,  $p < 0.04$ ).

### Group 3

**Clinical data.** The third part of the analysis examined data collected on the clinical behavior of the invasive disease in younger women (cases) and older women (controls) registered at the Ontario Cancer Treatment and Research Foundation, Kingston clinic, between 1950 and 1984. The mean age of the younger women was 30.2 (SE = 0.31) years and for older women 54.4 (SE = 0.86) years. The results in Table III indicate

a significant difference between younger and older women for stage of disease ( $\chi^2$  analysis = 19.0 with 5 df,  $p < 0.002$ ). Seventy-eight (64.5%) younger women and 104 (43.0%) older women presented with Stage I invasive disease, whereas 18 (14.9%) younger and 63 (26%) older women presented with Stage III or IV invasive disease.

Younger and older women presented with similar cell types and cell differentiation (Tables IV and V). Younger women differed significantly from older women in their treatment ( $\chi^2$  analysis = 37.12 with 5 df,  $p < 0.0001$ ). Younger women in the early stages tended to be treated by surgery significantly more often than the older patients with similar stages.

Overall survival for younger and older women is presented in Fig. 3. The results indicate a significant difference between younger and older women in prognosis, younger women having a better prognosis than

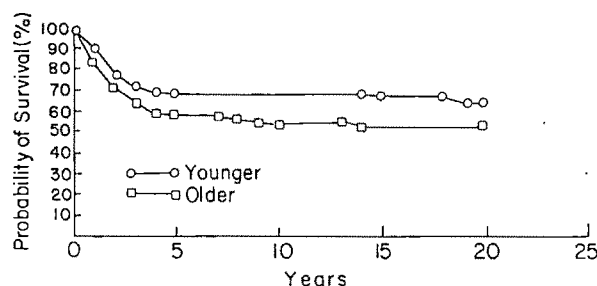


Fig. 3. Overall survival of all younger and older patients with invasive cervical carcinoma.

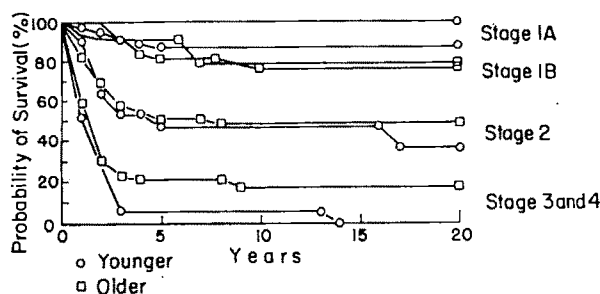


Fig. 4. Overall survival of younger and older women for all stages of invasive cervical carcinoma.

older women. Stage-by-stage survival is presented in Fig. 4. The results indicate no significant difference between younger and older women. Finally, shown in Figs. 5 and 6 is the prognosis for younger and older women diagnosed as having Stage IB invasive disease who underwent radical hysterectomy or radiation treatment. The results indicate no significant differences in prognosis by either mode of treatment between younger and older women.

## Comment

### Group 1

**Incidence data.** The results from the present study indicate little change in the age-adjusted incidence rates for Canada during the last decade. Increases were noted in the three prairie provinces. Provincial variation is difficult to explain but may be due in part to variation in screening activities and registration systems between the provinces. Despite the national experience, incidence data collected from the two local centers indicated an increase in the proportion of women 34 years of age and younger diagnosed as having invasive cervical carcinoma, particularly during the last decade.

### Group 2

**Cytologic data.** The cytologic histories of younger and older screened women indicated no significant difference in appropriateness of screening (for instance, the frequency of cytologic examination and appropriate response to abnormal cytologic report), frequency of

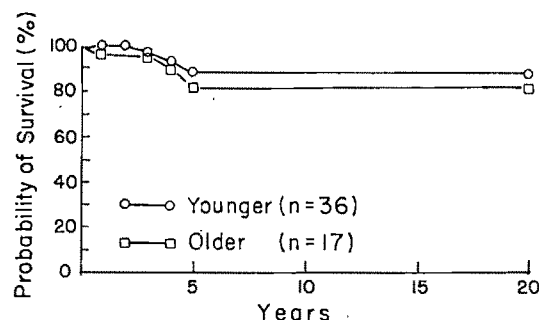


Fig. 5. Overall survival of younger and older women with Stage IB carcinoma treated by operation.

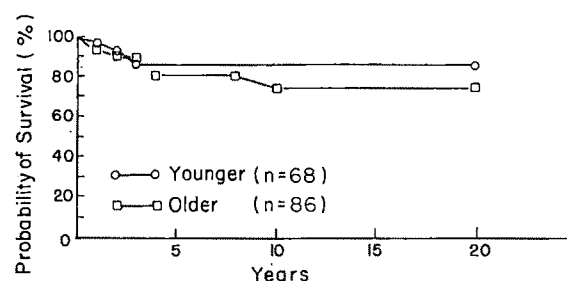


Fig. 6. Overall survival of younger and older women with Stage IB carcinoma who underwent pelvic irradiation.

overcalled cytologic results, and the time between the last negative cytologic smear and the diagnosis of invasive disease. Also, the stage distribution between younger and older women was similar. These findings are in contrast to observations made in the literature that indicate that younger women may be less susceptible to a screening program and that the natural history of the preinvasive disease may be shorter in this age group.<sup>1,3,7</sup> If younger women had a shorter preinvasive period it might be expected that they would present with a more advanced stage of disease. Fig. 2 shows this not to be the case, that is, when younger and older women had a negative smear within 3 years of diagnosis of the invasive disease the stage distribution was similar.

The single difference in the cytologic screening histories between younger and older women is the significant increase in undercalled cytologic reports in younger women. This finding is difficult to explain but has been reported elsewhere.<sup>7</sup>

### Group 3

**Clinical data.** Analysis of the clinical histories of the 363 women with invasive cervical carcinoma treated at the Ontario Cancer Treatment and Research Foundation, Kingston clinic, show that women 34 years of age and younger have a less advanced stage than women 35 years of age and older. This simply reflects the increased screening activity in the younger population.<sup>8</sup> The result of which is to lower the overall staging in the younger age group.<sup>9</sup>



The present study indicated a difference in overall survival between younger and older women, which can be explained simply on the basis of more younger women presenting with invasive disease in the earlier stages. The survival patterns are similar, stage by stage, for younger and older women. Different treatment modalities between younger women and older women with less advanced invasive disease in favor of surgery for younger women is an expression of the general trend to treat less advanced invasive disease, in this age group, with operation rather than radiation. However, when survival patterns are compared between younger and older women with Stage IB disease, survival patterns with radical hysterectomy treatment and radiation are similar for both age groups. Thus neither treatment modality favors younger women.

It is our opinion, on the basis of the present review, that there has been a recent slight increase in the proportion of younger women diagnosed as having invasive cervical carcinoma. The susceptibility to a screening program, the natural history of the disease, and the response to treatment are similar for younger and older women.

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## Biophysical profile scoring in the management of the postterm pregnancy: An analysis of 307 patients

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Management and outcome were reviewed in 307 consecutive postterm pregnancies assessed by biophysical profile scoring. Twice-weekly scores accurately differentiated normal fetuses from those at risk for intrauterine hypoxia. When the profile score is normal, waiting for spontaneous labor results in healthy neonates and a much lower cesarean section rate (15% versus 42% for "prophylactic" induction). Confident conservative management of postterm pregnancy is possible. (*AM J OBSTET GYNECOL* 1986;154:269-73.)

**Key words:** Postterm pregnancy, biophysical profile scoring

Pregnancy persisting beyond 42 weeks of gestation or 294 days from the first day of the last normal men-

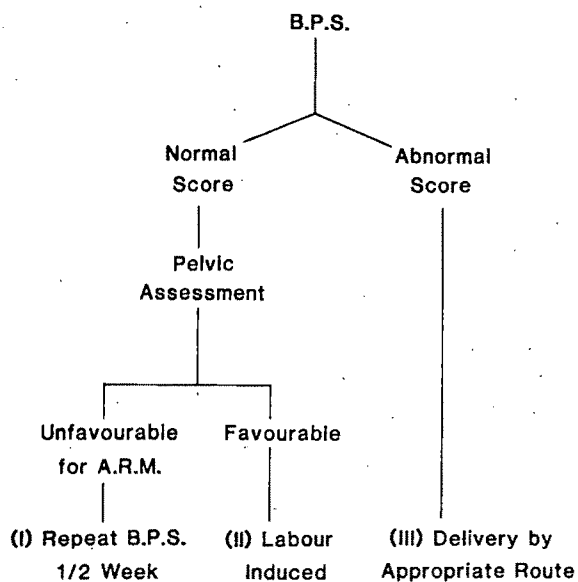
strual period is considered postterm and occurs in approximately 10% of all pregnancies.<sup>1</sup> Postterm pregnancy is associated with an increase in perinatal mortality, meconium-stained liquor, fetal distress in labor, and subsequent development and behavioral disturbances.<sup>2</sup>

Although it is a common problem, there is no unanimity of opinion regarding optimal management. Previous studies have reached little agreement as to when fetal jeopardy begins, how accurately the most endangered fetuses can be detected, or whether safe limits

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**Fig. 1.** Management protocol and outcome groups: (I), Normal biophysical profile scoring (B.P.S.), spontaneous labor (A.R.M., artificial rupture of membranes); (II), Normal biophysical profile scoring, labor induced; (III), Abnormal biophysical profile scoring, delivery by appropriate route.

can be established for the continuation of prolonged gestation.<sup>1-4</sup>

Because of this uncertainty, many centers continue to follow a policy of uniform delivery when 42 weeks of gestation is reached. The potentially serious complications of postmaturity in some (10% to 20%) is seen as justification for the possibly unnecessary intervention in the remaining 80% to 90% of pregnancies.

At our institution, antepartum fetal risk determination is based on a composite fetal biophysical profile score.<sup>3,6</sup>

The purpose of this study was to evaluate the benefits, if any, that are achieved when fetal biophysical profile scoring was used in the management of the patient with a postdate pregnancy. Two specific questions were addressed: (1) Can this method of assessment accurately detect the fetus at risk of intrauterine compromise because of postmaturity? (2) If the fetus continues to appear healthy according to the biophysical profile scoring, does withholding intervention beyond 42 weeks result in improved maternal outcome? If this practicable means of fetal assessment can accomplish both accurate intervention when the fetus is jeopardized and confident conservatism that protects the mother from the consequences of unnecessary intervention when the fetus is healthy, then its application in prolonged gestation would be validated.

#### Material and methods

Criteria for inclusion in this prospective management study were as follows: single pregnancies with no

other complications, with firmly established gestational length of more than 294 days from the known last normal menstrual period. Dates were confirmed in all patients by early examination or early ultrasound studies or by both, and patients were excluded if the ultrasound findings on referral to the study group resulted in reassignment of dates.

Biophysical profile scoring as described by Manning et al.<sup>6</sup> was performed twice weekly on all patients. If all four ultrasound parameters (fetal movement, muscle tone, fetal breathing movements and amniotic fluid volume) were present, the biophysical profile scoring was considered to be normal, and a nonstress test was not done (biophysical profile scoring of 8/8'). If any one of the first three ultrasound parameters listed was not present, a nonstress test was given, and if reactive, the biophysical profile scoring was again considered normal (biophysical profile scoring of 8/10). For scores of 6/10 or 8/10 in which amniotic fluid volume was the abnormal variable (pocket of <2.0 cm in vertical dimension, measured in two planes), the score was considered abnormal.

Based on the biophysical profile score obtained at each visit, the patient was managed according to the protocol illustrated in Fig. 1.

Three outcome groups were generated by the study (Table I). Group I consisted of patients with normal biophysical profile scoring, followed without intervention until the spontaneous onset of labor. Group II patients also had normal scores until labor, which was induced with a cervix favorable for artificial rupture of membranes. Group III consisted of those in whom an abnormal score suggesting fetal compromise constituted a definite indication for delivery, which was undertaken within 24 hours by the appropriate route. For the purposes of analysis, group III was subdivided according to the depth of the largest amniotic fluid pocket available.<sup>8</sup>

A fourth group of patients was generated because the attending physicians did not always follow the recommendations of the protocol. These patients all had a normal score at their last assessment but labor was induced "for dates only" despite a cervix unfavorable for artificial rupture of the membranes (Group IV).

In all cases the last biophysical profile scoring before delivery was the score of preference in the analysis. Parameters of fetal perinatal morbidity were defined as (1) fetal distress in labor requiring emergency cesarean section, (2) 5-minute Apgar score of  $\leq 6$ , and (3) meconium aspiration requiring admission to neonatal intensive care. Apgar scores were assigned by independent observers. Intubation and suctioning were performed by a neonatologist or anesthetist on all infants with meconium present in amniotic fluid. The rate of cesarean section was calculated for each group.

**Table I.** Management/outcome groups (n = 293)

Group	Biophysical profile scoring	Description of delivery	n	Mean birth weight (gm)	Mean gestational length (days)
I	Normal	Spontaneous labor	180	3997	301
II	Normal	Induced labor/favorable cervix	31	3853	302
III	Abnormal	Delivered because of fetal indications	32	3664	298
IV	Normal	Induced for dates only	50	3799	301

**Table II.** Incidence of perinatal morbidity

Morbidity	Group I		Group II		Group III		Group IV	
	n	%	n	%	n	%	n	%
Cesarean section for fetal distress	6	3.3	1	3.2	7	22*	7	14†
5 min Apgar score of ≤6	3	1.6	1	3.2	4	12.5*	1	2
Meconium aspiration to intensive care nursery	4	2.2		0	6	19*		0

\*Significantly different from all other groups,  $p < 0.05$  by  $\chi^2$ .

†Significantly different from groups I and II,  $p < 0.05$  by  $\chi^2$ .

The four management/outcome groups were compared with use of  $\chi^2$  contingency tables or Student's  $t$  test as was appropriate. A value of  $p < 0.05$  was considered significant.

### Results

Over the 3-year period beginning September 1, 1981, 307 women referred for composite fetal assessment met the entry criteria. Fourteen of these women were delivered because of changes in maternal condition not related to postdatism and were excluded from further analysis. This resulted in a final study population of 293 patients, who had a total of 503 assessments.

These patients were distributed among the four outcome/management groups, as shown in Table I. While differing in number, these groups did not differ significantly in distribution of maternal age, parity, or birth weight. Although patients in group III tended to require delivery sooner (for abnormal biophysical profile scoring that suggested fetal compromise) the range of time beyond 42 weeks at which the scoring became abnormal (0 to 12 days) rendered this difference statistically nonsignificant (Student's  $t$  test). All fetuses in group III were delivered within 24 hours of their abnormal biophysical profile scoring, whereas the majority of all other groups delivered within 4 days of their normal, last profile scoring.

There were no stillbirths or perinatal deaths in the entire study population. There were significant differences in perinatal morbidity between groups (Table II). Fetuses in group III required cesarean section for fetal distress more often than any other group and had a total cesarean section rate of 37.5%. Fetuses in group III (delivered for abnormal biophysical profile scoring) accounted for 44% of low 5-minute Apgar scores and for 60% of neonatal admission to intensive care for

**Table III.** Cesarean section following normal biophysical profile scoring

Group	Cesarean section for fetal distress		Total cesarean sections	
	n	%	n	%
I. Spontaneous labor	6	3.3	27	15
II. Induced labor favorable cervix	1	3.2	4	13
IV. Induced for dates only	7	14*	21	42*

\*Significantly different from groups I and II,  $p < 0.01$  by  $\chi^2$ .

meconium aspiration. The frequency of cesarean delivery for fetal distress was also increased in group IV, but the frequency of low Apgar scores and serious meconium aspiration were not.

Within group III (32 patients) perinatal morbidity was highest in fetuses with the lowest amniotic fluid volume. Among the 11 with overt oligohydramnios (largest pocket of fluid  $< 1.0$  cm) four required cesarean section for fetal distress, eight had meconium in amniotic fluid, and six had meconium below their vocal cords, necessitating admission to the intensive care nursery for three of them. Six of the 11 had low Apgar scores at 1 minute and two at 5 minutes after birth. The nine fetuses with marginal fluid (pocket of 1 to 2 cm) had an average amniotic fluid volume of 1.6 cm. In this subgroup, two required delivery by cesarean section (there were no low 5-minute Apgar scores), and one was admitted to the intensive care nursery for meconium aspiration. In the subgroup with normal ( $> 2.0$  cm) amniotic fluid volume but abnormal biophysical profile scoring, six of 12 required cesarean section, two had low Apgar scores at 5 minutes, and two were ad-



**Table IV.** Collective neonatal morbidity

	Group I		Group II		Group III		Group IV	
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
Neonatal morbidity (No. of babies affected)	7	3.8	1	3.2	6	18.7	2	4

mitted to the intensive care nursery. This last subgroup included one fetus with microcephaly born to a mother with untreated phenylketonuria.

Significantly different cesarean section rates were observed between the groups delivered after a normal final biophysical profile score (Table III). In each of these groups the majority of cesarean sections performed were for cephalopelvic disproportion and failure to progress. The patients in whom labor was induced despite an unfavorable cervix (group IV) constituted 19.2% (50 of 261) of those delivered following a normal biophysical profile scoring but accounted for 40.4% (21 of 52) of cesarean sections in that population. The cesarean section rate for patients with normal biophysical profile scoring managed according to protocol (groups I plus group II) was 14.7% (31 of 211) compared to 42% (21 of 50) for group IV ( $p < 0.005, \chi^2$ ). Among the cesarean sections in group IV, at least four were primary elective procedures on the combined basis of an unfavorable cervix, clinically large baby, and postdatism.

During the period of this study the hospital's overall cesarean section rate was 16.5%.

#### Comment

The appropriate management of the obstetric patient whose gestation has exceeded 42 completed weeks (postmaturity or postdates syndrome) remains one of the most difficult problems in modern perinatal medicine. The perinatal risks of the postdates syndrome are well established; perinatal mortality doubles for each additional week after the forty-second week.<sup>9</sup> One simple solution to this problem would be to deliver all patients by the end of the forty-second week. However, such a nonselective approach, while being done to prevent perinatal morbidity and mortality, may create iatrogenic morbidity for the mother. In this present study, when this nonselective approach to timing of delivery was used, the cesarean section rate rose to 42%, more than 2.5 times the rate for the general population at our institution (16.5%).

A more logical approach may be to consider both fetal and maternal prognostic factors in selecting the most appropriate management strategy. Fetal biophysical profile scoring, as measured in a study population of 12,620 high-risk patients, has proved to be a very accurate method of determination of fetal well-being

at risk.<sup>6</sup> When this method was applied to this special-risk category of postdate pregnancies, it appeared also to be of value in assigning fetal risk. In those fetuses with normal biophysical activities and normal amniotic fluid volume ( $n = 211$ ), who were managed according to our protocol (Fig. 1), there were no perinatal deaths or fetal distress, low Apgar scores were infrequent (3.31% and 1.89%, respectively), and subsequent neonatal morbidity was unusual (1.9%)(Table II). In contrast, in those fetuses exhibiting an abnormal biophysical profile score and/or oligohydramnios ( $n = 32$ ), the incidence of fetal distress (22%), low Apgar scores (12.5%), and neonatal morbidity (19%) were all substantially and significantly increased (Table II). When considered collectively, neonatal morbidity ranged from 3.7% when the fetal biophysical profile score was normal to 18.7% when the score was abnormal (Table IV). These data indicate that fetal biophysical profile scoring facilitates differentiation of the normal noncompromised fetus from the compromised fetus within a population of postdates pregnancies. Accurate recognition of fetal risk, when in turn combined with maternal obstetric assessment (including cervical findings), allows for a rational and selective approach to patient care. The potential beneficial impact of such a selective approach in reducing maternal morbidity is clear. In this present study some of this benefit was realized. The cesarean section rates for patients with a normal fetus managed conservatively and those with a normal fetus delivered because of favorable cervical findings were similar (15% and 13%, respectively) and were not increased as compared to the population at large (16.5%). In contrast, both were sharply and significantly lower than the cesarean section rate observed in patients induced in a nonselective manner on the basis of gestational age alone (42%)(Table III).

The results of this prospective clinical study of a selective management strategy for the postdates pregnancy are very encouraging. In study patients perinatal mortality was absent and morbidity was low while intervention rates were reduced, at least as compared to those observed among patients with nonselective intervention based on gestational age alone. Based on these findings we would suggest that it may no longer be reasonable to elect routine delivery of all patients at or beyond 42 completed weeks of gestation. In view of the

proven reliability of fetal assessment methods and the potential risk of nondiscriminative intervention, selective patient care appears to be the method of choice.

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## Occurrence of molar pregnancy in patients undergoing elective abortion: Comparison with other clinical presentations

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Clinical data of molar pregnancies found in women undergoing elective abortion (group 1,  $n = 39$ ) were compared to those of molar pregnancies in women who experienced spontaneous abortions (group 2,  $n = 157$ ) and women in whom molar pregnancy was discovered before symptoms of spontaneous abortion were evident (group 3,  $n = 209$ ). Group 1 women were younger and experienced uterine evacuation at an earlier stage of amenorrhea than groups 2 and 3. Group 3 had larger uteri at evacuation and longer intervals of positive tests for the  $\beta$ -subunit of human chorionic gonadotropin during the postmolar phase as compared with groups 1 and 2. On the basis of available provincial data for the number of elective abortions, the estimated incidence of molar pregnancies in this population was 1:2,699. The presence of malignant gestational trophoblastic neoplasia was documented in a single patient in group 1. The incidence of malignant gestational trophoblastic neoplasia in this group was not significantly different from that in groups 2 and 3. Routine pathologic examination of the products of conception in women undergoing elective abortion coupled with routine assays of the  $\beta$ -subunit of human chorionic gonadotropin when molar pregnancy is found can identify both noninvasive and invasive trophoblastic disease in these women. (*AM J OBSTET GYNECOL* 1986;154:273-6.)

**Key words:** Molar pregnancy, elective abortion, spontaneous abortion

Currently, there is little information about the frequency and clinical behavior of molar pregnancy found in women presenting for elective abortion. In this ar-

ticle we report on 39 patients with molar pregnancy discovered by routine pathologic examination of the products of conception from patients undergoing elective abortion in British Columbia between 1975 and 1984. The clinical course and regression patterns of the  $\beta$ -subunit of human chorionic gonadotropin ( $\beta$ -hCG) of these patients after molar pregnancy were contrasted to those in patients with molar pregnancies found by pathologic examination in women with spontaneous abortions and in women in whom molar pregnancies were discovered before symptoms and signs of spontaneous abortion occurred.

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**Table I.** Clinical and  $\beta$ -hCG regression data in women with molar pregnancy undergoing elective abortion (group 1), women experiencing spontaneous clinical abortion (group 2), and in women in whom molar pregnancy was diagnosed without symptoms or signs of spontaneous abortion (group 3)

Group	Age (yr)	Gravidity	Gestational age (wk)	Uterine size (wk)	$\beta$ -hCG (days to negative, <5 mIU/ml)
1 (n = 39)	23.2 $\pm$ 1*	2.0 $\pm$ 0.3	8.9 $\pm$ 0.3*	10.2 $\pm$ 0.5	57.2 $\pm$ 7.4
2 (n = 157)	26.5 $\pm$ 0.6	2.2 $\pm$ 0.1	13.0 $\pm$ 0.6	10.2 $\pm$ 0.3	59.6 $\pm$ 4.9
3 (n = 209)	26.2 $\pm$ 0.4	2.2 $\pm$ 0.1	14.4 $\pm$ 0.5	14.9 $\pm$ 0.5*	79.21 $\pm$ 0.8*

Data are shown as mean  $\pm$  SEM. The gestational age and uterine size are in weeks of amenorrhea.

\*p < 0.05, as compared with other groups (analysis of variance followed by Duncan's multiple range test).

**Table II.** Incidence of malignant trophoblastic neoplasia developing in groups 1, 2, and 3 (as defined in Table I)

Group	Malignant trophoblastic neoplasia (n)	Molar pregnancy (n)	Ratio	% Malignant trophoblastic neoplasia
1	1	39	1:39	2.56
2	3	157	1:52	1.9
3	19	209	1:11	9.09

The incidence of malignant trophoblastic neoplasia developing in group 1 was not significantly different from that in groups 2 and 3, Fisher's exact test.

**Table III.** Clinical and  $\beta$ -hCG regression data (mean  $\pm$  SEM) in 23 women who developed malignant gestational neoplasia requiring chemotherapy

Age (yr)	Gravidity	Gestational age (wk)	Uterine size (wk)	$\beta$ -hCG (days to negative, <5 mIU/ml)
25.3 $\pm$ 0.8	1.9 $\pm$ 0.2	12.4 $\pm$ 0.5	15.3 $\pm$ 1	181.1 $\pm$ 21.1

### Material and methods

Patients were identified from a molar pregnancy registry as previously described.<sup>1,2</sup> Only patients with classical (complete) moles were included in the data presented. The postmolar phase was monitored by serial radioimmunoassays of the plasma concentrations of the  $\beta$ -hCG as previously described.<sup>1,2</sup> Briefly, samples were assayed weekly until there were three consecutive negative values and then at 2- to 4-week intervals until tests were negative for an interval of at least 6 months. Malignant gestational trophoblastic neoplasia was suspected and chemotherapy considered when  $\beta$ -hCG levels plateaued or persistently increased during at least a 3-week period.

Thirty-nine patients with molar pregnancy discovered by routine pathologic examination of the products of conception, after elective abortion in the years 1975 to 1984, formed group 1. Group 2 consisted of 157 women with molar pregnancy found after the onset of clinical symptoms of spontaneous abortion (bleeding and/or passage of tissue). In this group, tissue submitted for pathologic examination included material passed through the vagina and material obtained at the time of uterine curettage. Group 3 consisted of 209 women with molar pregnancy discovered before symp-

toms of spontaneous abortion were evident (bleeding and/or passage of tissue); subsequent uterine evacuation confirmed the molar pregnancy in all patients. All elective abortions in group 1, uterine curettage procedures when indicated in group 2, and evacuations of molar pregnancy in group 3 patients were performed in the hospital and the products of conception were routinely examined by a certified pathologist. Tissue from each patient was submitted to histologic examination. Data on the number of elective abortions were provided by the Ministry of Health, Province of British Columbia. Data in Table I were first analyzed by the analysis of variance. Where an overall difference was found, Duncan's multiple range test was then used to determine the differences between the means noted. Data in Table II were analyzed with the use of Fisher's exact test (group 1 versus group 2 and group 1 versus group 3). Differences were considered significant when p was <0.05.

### Results

As shown in Table I, patients in group 1 were significantly younger and their mean gestational ages were significantly less than those of the other groups. Group 3 patients had significantly larger uteri at the time of

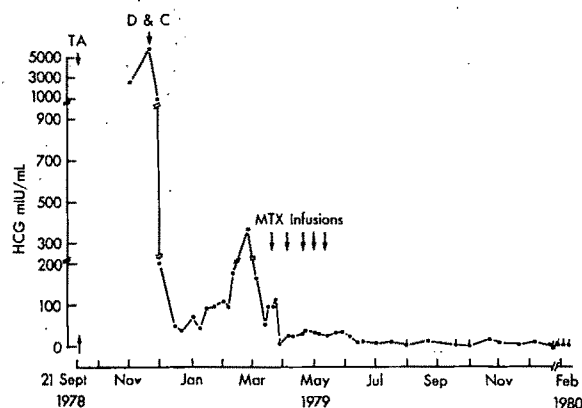
uterine evacuation and the duration of positive tests for  $\beta$ -hCG was also significantly longer than that of the other two groups.

Complete provincial statistics were available for the number of patients undergoing elective abortion between 1975 to 1982 inclusive. During this 8-year interval there were 97,165 elective abortions in the province and 36 molar pregnancies in this group were detected. The estimated incidence of molar pregnancy in elective abortions (which are legally permitted up to 20 weeks of amenorrhea) was one molar pregnancy per 2699 elective abortions. During the study interval (1975 to 1984 inclusive) there were 23 women who developed malignant gestational trophoblastic neoplasia during the interval after molar pregnancy. Clinical and  $\beta$ -hCG data are shown in Table III. The incidence of malignant gestational trophoblastic neoplasia within the three subgroups of molar pregnancies is shown in Table II. The variation in incidence of malignant gestational trophoblastic neoplasia in group 1, as compared to groups 2 and 3, was not statistically significant.

The patient in group 1 who developed malignant trophoblastic neoplasia was a 24-year-old unmarried woman with an unwanted pregnancy. Elective abortion was undertaken by suction curettage at 12 weeks of gestation. The presence of complete (classical) molar pregnancy was found on histologic examination. There were large hydropic avascular villi of variable sizes with moderate trophoblastic proliferation. Following the abortion, Ortho-Novum 1/80-21 was used for contraception. Continued bleeding in the postevacuation interval together with persistently elevated plasma  $\beta$ -hCG levels prompted a further curettage, which confirmed persistent molar tissue in the uterus. The clinical events and  $\beta$ -hCG levels are summarized in Fig. 1. Because of a persistent and rising trend in the  $\beta$ -hCG levels during the postevacuation interval, a diagnosis of nonmetastatic malignant gestational trophoblastic disease was made. Six months after the termination of pregnancy, five courses of intravenous methotrexate therapy with folinic acid rescue during a 2-month period (Fig. 1) successfully induced resolution of the malignant gestational trophoblastic neoplasm in this patient.

### Comment

During the 10-year period 1975 to 1984 inclusive, 39 patients had molar pregnancies found by the routine pathologic examination of the evacuated products of conception after undergoing elective abortion. One of these women later developed malignant gestational trophoblastic neoplasia, which responded to chemotherapy. During the 8-year period between 1975 and 1982, when complete provincial statistics for the number of elective abortions were available, there were 36 molar



**Fig. 1.** In this patient elective abortion (TA) was followed by repeat uterine curettage (D & C). The initially rising  $\beta$ -hCG levels ( $\beta$ -subunit assay, second international standard) declined rapidly after curettage, then gradually showed a persisting and increasing trend. Six months after the elective abortion, five courses of treatment with high-dose intravenous methotrexate (MTX) followed by folinic acid rescue were given during a 2-month period. The dose of MTX was 500 mg/m<sup>2</sup> of surface area (total dose 810 mg) per course of treatment. The vertical bars above certain data points indicate  $\beta$ -hCG levels below the limit of assay sensitivity (5 mIU/ml). Data are shown to February, 1980. Subsequent follow-up (data not shown) confirmed remission to October, 1980.

pregnancies among 97,165 elective abortions. The estimated incidence of molar pregnancy in women undergoing elective abortion was one in 2699. In a comparison of the clinical outcome it was apparent that group 1 patients experienced a relatively benign postmolar course; however, invasive complications can occur and the incidence of these complications, although low, was not statistically different from the other traditional clinical presentations of molar pregnancy represented by groups 2 and 3 (Table II). Nevertheless, among the three subgroups studied, group 3 had the highest rate of invasive complications. These women presented later in the course of molar pregnancy and as a result the uteri were larger at presentation. During the postmolar interval the  $\beta$ -hCG levels remained positive for longer periods (Table I) and 9.1% of this group developed malignant trophoblastic neoplasia as compared with 2.6% and 1.9% for groups 1 and 2, respectively. In contrast, women in group 1 were younger and at evacuation of the uteri they had experienced a shorter interval of amenorrhea as compared with that of groups 2 and 3 (Table I).

Cohen et al.<sup>3</sup> described eight patients with hydatidiform mole among 4829 patients presenting for elective first-trimester abortions at The Johns Hopkins University medical institutions in Baltimore, Maryland. The incidence of molar pregnancy in their population was one in 600 patients undergoing first-trimester elec-



tive abortion. Most of these women were black (six of eight, or 75%), less than 16 years of age, and apparently of diminished socioeconomic status. The patients we studied differed in their demographic features. The ethnic background in group 1 was estimated in 28 of the 39 women in whom this was known. Only one woman (3.57%) was black, 23 (82.14%) were Caucasian, three (10.71%) were Asian (Chinese and Japanese), and the remaining woman (3.57%) was North American Indian. As shown in Table I the women we studied were also older, with a mean age of 23.2 years. In addition, although all the patients with molar pregnancy in group 1 underwent evacuation in the first trimester of pregnancy, we expressed our results in terms of all women undergoing elective abortion in the province. Legally this interval is up to 20 weeks of amenorrhea. These factors probably explain the differences in the frequency of molar pregnancy described in women undergoing elective abortion in British Columbia and Baltimore.<sup>3</sup>

In summary, during a 10-year period we identified 39 patients with molar pregnancy in a population of women undergoing elective abortion in British Columbia. Using available provincial statistics for the number of elective abortions during an 8-year period of the study we estimated that one of 2699 otherwise asymptomatic women undergoing elective abortion in the province had a molar pregnancy. These women (group 1), were identified by routine pathologic examination of the evacuated products of conception. Although their clinical courses were relatively benign, they were

also younger, and the uteri were evacuated at an earlier period of amenorrhea. The incidence of malignant trophoblastic neoplasia developing in this group was not significantly different from that in women found to have a molar pregnancy after presenting with clinical symptoms and signs of spontaneous abortion (group 2) or that in women in whom the diagnosis of a molar pregnancy was made before clinical evidence of spontaneous abortion was present (group 3). Routine pathologic examination of the products of conception in women undergoing elective abortion coupled with routine  $\beta$ -hCG assays when molar pregnancy is found can identify both noninvasive and invasive trophoblastic disease in these women.

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# Maternal serum $\alpha$ -fetoprotein and fetal autosomal trisomies

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Maternal serum  $\alpha$ -fetoprotein values in 61 patients with fetal autosomal trisomies diagnosed at 6581 genetic amniocenteses were significantly lower than those in an equal number of matched control subjects. The genetic risk of fetal autosomal trisomies for women <age 35 offered amniocentesis with maternal serum  $\alpha$ -fetoprotein values <0.5 multiples of the median is in the same range as that currently accepted for advanced maternal age. Maternal serum  $\alpha$ -fetoprotein screening for autosomal trisomies (mainly Down syndrome) is feasible. (AM J OBSTET GYNECOL 1986;154:277-81.)

**Key words:**  $\alpha$ -Fetoprotein, fetal trisomy, amniocentesis

Merkatz et al.<sup>1</sup> were the first to discover an association between low maternal serum  $\alpha$ -fetoprotein values and fetal autosomal trisomies. Several populations were studied and each pregnancy associated with an infant with a chromosomal abnormality was randomly matched with two control pregnancies according to maternal age and weeks of gestation at the time of  $\alpha$ -fetoprotein testing for matching. Of the 44 cases of aneuploidy, only nine of the women had serum values at or above the median. This distribution differed significantly from that of the control subjects, among whom 48 had values below the median and 40 had values at or above the median. In six (14%) of the 44 cases of aneuploidy compared with 5.3% of the controls, values were <0.25 multiples of the median. Similarly, in 11 (25%) of the 44 cases of aneuploidy compared with 11.2% of controls, maternal serum  $\alpha$ -fetoprotein values were <0.4 multiples of the median.

In a further study by Cuckle et al.<sup>2</sup> results were similar. Serum  $\alpha$ -fetoprotein results were available on 61 affected pregnancies with Down syndrome. The samples were obtained between 14 and 20 weeks' gestation and all  $\alpha$ -fetoprotein results were expressed as multiples of the median. A control group was made up of 36,652 singleton unaffected pregnancies in which maternal serum  $\alpha$ -fetoprotein screening had been done. The study showed that the median maternal serum  $\alpha$ -fetoprotein value for Down syndrome fetuses was significantly lower than normal (0.72 multiples of the

median); 49 of 61 values were below the median and only 12 of 61 above.

Both groups suggested that this finding indicated the potential for assay of serum  $\alpha$ -fetoprotein as a valuable screening test for fetal Down syndrome. These two articles prompted a retrospective review of maternal serum  $\alpha$ -fetoprotein values in our own patients at high genetic risk, with pregnancies in which fetal autosomal trisomies had been diagnosed. We also reviewed our regional pilot serum  $\alpha$ -fetoprotein screening project data with particular emphasis on low serum  $\alpha$ -fetoprotein values and neonates with Down syndrome delivered of mothers who had been screened.

## Material and methods

A total of 6851 genetic amniocenteses were performed from 1978 to December 31, 1984, in our regional prenatal genetic diagnosis program. A total of 61 fetuses with autosomal trisomies were diagnosed during that period: 46 with trisomy 21, 10 with trisomy 18, and five with trisomy 13.

In each of these patients, the maternal serum  $\alpha$ -fetoprotein assay had been done by radioimmunoassay immediately before genetic amniocentesis. A control group was also analyzed for maternal serum  $\alpha$ -fetoprotein results. This group consisted of the next patient with the same gestational age who underwent amniocentesis immediately after the patient in whom a fetal autosomal trisomy had been diagnosed. Because of the change in serum  $\alpha$ -fetoprotein standard levels during 1982, values were expressed in multiples of the median for a comparison of the maternal serum  $\alpha$ -fetoprotein levels in trisomy and control groups.

A pilot serum  $\alpha$ -fetoprotein project in Southern Ontario involving normal (low genetic risk) prenatal patients was begun in May, 1982. The maternal serum  $\alpha$ -fetoprotein samples were taken at 16 to 18 weeks'

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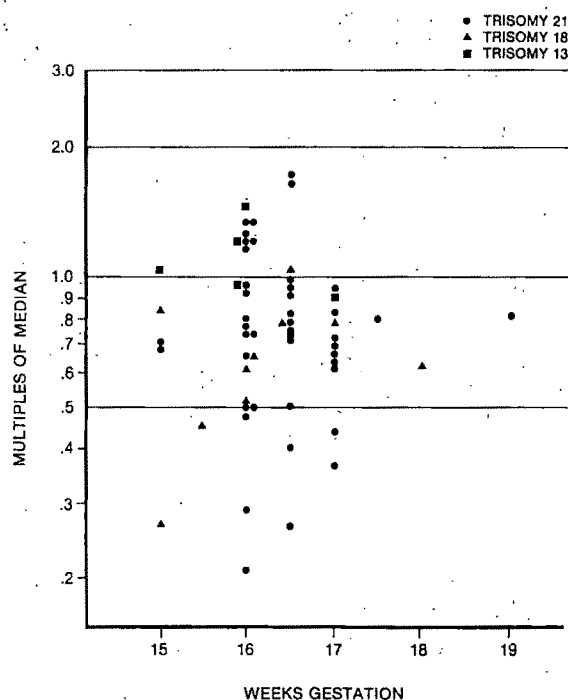


Fig. 1. Serum  $\alpha$ -fetoprotein values in multiples of the median for 61 patients with fetal autosomal trisomies diagnosed at amniocentesis.

gestation and if the level was abnormal, that is, high ( $>2$  times the median) or low ( $<0.25$  times the median), an ultrasound examination was done and another sample was obtained. Patients with "unexplained" high values were offered a level 2 detailed ultrasound examination and amniocentesis for amniotic fluid  $\alpha$ -fetoprotein and acetylcholinesterase assay. From May, 1982, to September 30, 1984, a total of 6505 patients underwent serum  $\alpha$ -fetoprotein screening, and pregnancy outcome has been fully documented in 1854 of these patients. In this latter group, there were three women who were delivered of infants with Down syndrome (trisomy 21).

## Results

**High genetic risk population (genetic amniocentesis group).** The maternal serum  $\alpha$ -fetoprotein results in the 61 pregnancies associated with fetal autosomal trisomy and in control patients are given in Table I and Figs. 1 and 2. In 12 of 61 (19.7%) fetuses with autosomal trisomies compared with one of 61 (1.6%) fetuses in control patients, maternal serum  $\alpha$ -fetoprotein levels were  $<0.5$  multiples of the median. Similarly, in 46 of 61 (78.6%) fetuses with autosomal trisomy compared with 32 of 61 (52%) fetuses in control patients, maternal serum  $\alpha$ -fetoprotein values were less than the median. The maternal serum  $\alpha$ -fetoprotein values in cases of trisomy were 0.80 multiples of the median compared with 0.99 multiples of the median in control pregnancies.

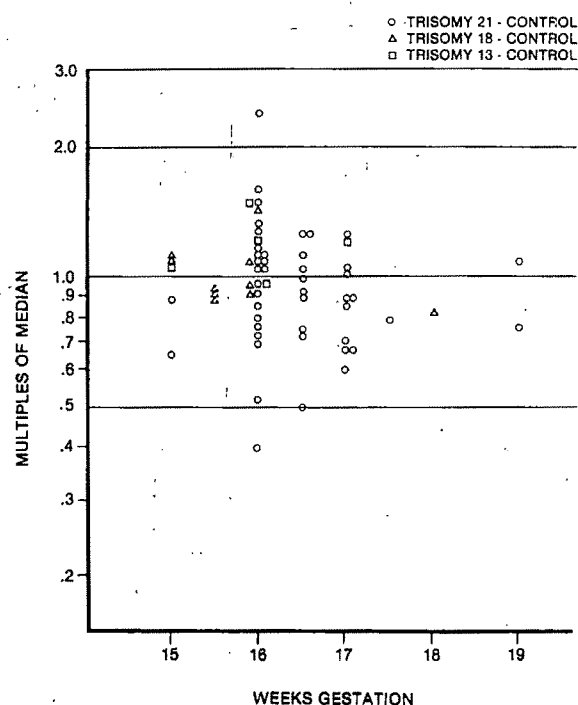


Fig. 2. Serum  $\alpha$ -fetoprotein values in multiples of the median for 61 control patients with normal results at amniocentesis.

Similarly, in two of the subgroups, maternal serum  $\alpha$ -fetoprotein values expressed in multiples of the median were lower in cases of trisomy 21 (0.79 versus 0.97,  $p < 0.01$ ) and trisomy 18 (0.64 versus 1.01,  $p < 0.001$ ). In trisomy 13 (five patients) the mean maternal serum  $\alpha$ -fetoprotein level was not lower than that in the control subjects (1.19 versus 1.18). Amniotic fluid data from this study in both the autosomal trisomy patients and the control subjects are summarized in Table II. These results are expressed in multiples of the mean rather than in multiples of the median because of the construction of our normal range for amniotic fluid  $\alpha$ -fetoprotein, which is the mean  $+ 5$  SD. Amniotic fluid  $\alpha$ -fetoprotein values in fetal trisomy 21 and trisomy 18 and in all autosomal trisomies combined were less than those in control patients. However, values in trisomy 13 were not lower than those in control pregnancies.

**Low-risk population (serum  $\alpha$ -fetoprotein screening project).** The clinical and laboratory data of the three women who were delivered of infants with trisomy 21 are summarized in Table III. One can see from inspecting these results that two of three maternal serum  $\alpha$ -fetoprotein results were  $<0.5$  times the median.

## Comment

A comparison of our data and those of other published studies regarding fetal autosomal trisomies and maternal serum  $\alpha$ -fetoprotein values is given in Table

**Table I.** Summary of maternal serum  $\alpha$ -fetoprotein values (expressed in multiples of median) in fetal autosomal trisomies

Trisomy	n	Mean MSAFP (MoM)	<0.05 MoM		<5th Percentile		<10th Percentile	
			No.	%	No.	%	No.	%
Trisomy 21	46	0.79	10/46	21.7	7/46	15.2	11/46	23.9
Control	46	0.97	1/46	2.2	1/46	2.2	3/46	6.5
p Value		<0.01						
Trisomy 18	10	0.64	2/10	20	1/10	10	3/10	30
Control	10	1.01	0/10	0	0/10	0	0/10	0
p Value		<0.001						
Trisomy 13	5	1.19	0/5	0	0/5	0	0/5	0
Control	5	1.18	0/5	0	0/5	0	0/5	0
p Value		NSD						
All trisomies	61	0.80	12/61	19.7	8/61	13.1	14/61	23.0
All controls	61	0.99	1/61	1.6	1/61	1.6	3/61	4.9
p Value		<0.005						

MSAFP = Maternal serum  $\alpha$ -fetoprotein; MoM = multiples of the median; NSD = not significantly different.

**Table II.** Summary of amniotic fluid  $\alpha$ -fetoprotein values (expressed in multiples of mean) in fetal autosomal trisomies

Trisomy	n	Mean $\alpha$ -fetoprotein (multiples of mean)
Trisomy 21	46	0.62
Control	46	1.01
p Value		<0.001
Trisomy 18*	10	1.51
Control*	10	0.93
Trisomy 13†	5	1.07
Control†	5	1.12
All trisomies	61	0.81
All controls	61	1.01
p Value		<0.02

\*With the *t* test, there was not significant difference between the means of the two samples.

†With the *t* test, there was no significant difference between the means of the two samples.

IV. Our results are in agreement with the majority of the results in the literature, that is, that maternal serum  $\alpha$ -fetoprotein values are significantly lower in association with Down syndrome fetuses. Most studies, including our own, found that approximately 20% of values were <0.5 multiples of the median with Down syndrome fetuses and in fetal autosomal trisomies combined (13, 18, and 21).<sup>1-6</sup> The only published study that did not find this association was that of Cowchuk and Rush,<sup>7</sup> and in this study, the serum  $\alpha$ -fetoprotein assays were done on serum samples that had been frozen and stored for some time (average 3 months). Although this should not have had an effect on their results, such an effect seems possible.

We selected an arbitrary cutoff point of 0.5 multiples of the median for comparative purposes because this point was used by many authors as a suggested cutoff

**Table III.** Neonates with trisomy 21 in serum  $\alpha$ -fetoprotein screening project

Patient No.	Maternal age (yr)	Gestational age at MSAFP determination (wk)	MSAFP ( $\mu$ g/L)	Result (MoM)
42	26	17	17	0.43
260	36	15	9.3	0.39
255	29	16	29	0.94

MSAFP = Maternal serum  $\alpha$ -fetoprotein; MoM = multiples of the median.

for defining low maternal serum  $\alpha$ -fetoprotein values and was one for which the percentage of a normal prenatal population that would have to be screened to detect Down syndrome fetuses and other trisomies was in an appropriate range, that is, less than 8%. The percentage of maternal serum  $\alpha$ -fetoprotein values <0.5 multiples of the median in a normal prenatal population in any particular study is important and varied between various centers from 3.7% to 8%. Cuckle et al.<sup>2</sup> reduced this from 8.4% to 5% on the basis of revision of gestational age by ultrasound and this seems to be a valid approach.

Although specific maternal serum  $\alpha$ -fetoprotein data on fetal trisomies 18 and 13 are available only from our study and that of Merkatz et al.,<sup>1</sup> numbers are small. There is an indication from these two studies that maternal serum  $\alpha$ -fetoprotein values in trisomy 18 are similar to those in Down syndrome (lower than normal). In trisomy 13, however, the evidence to date is not convincing for any difference in maternal serum  $\alpha$ -fetoprotein values. Our findings of decreased amniotic fluid  $\alpha$ -fetoprotein levels with fetal Down syndrome are in accordance with those of most other authors.<sup>2,4,7</sup> The concept that this represents decreased



**Table IV.** Comparison of maternal serum  $\alpha$ -fetoprotein values and relation to fetal autosomal trisomies in published studies

Study	Merhatz <i>et al.</i> <sup>1</sup>	Cuckle <i>et al.</i> <sup>2</sup>	Doran <i>et al.</i> (this study)	Fuhrman <i>et al.</i> <sup>3</sup>	Tabor <i>et al.</i> <sup>4</sup>	Seller <sup>5</sup>
Trisomy 21						
MSAFP MoM	—	0.72	0.79	—	0.75 (25)	0.8
MSAFP <0.5 MoM	7/25 (28%)	13/61 (21%)	10/46 (21.7%)	9/43 (21%)	—	1/8 (22%)
MSAFP control <0.5 MoM	5.0%	8.4 → 5.0%	7.0%	3.7%	—	7.0%
AFAFP	Normal	0.6 (MoM)	0.62 (MoX)	—	0.64 (MoM)(25)	—
Trisomy 18						
MSAFP MoM	—	—	0.64	—	—	—
MSAFP <0.5 MoM	3/13 (23%)	—	2/10 (20%)	—	—	—
MSAFP control <0.5 MoM	5.0%	—	7.0%	—	—	—
AFAFP	—	—	1.51 (MoX)	—	—	—
Trisomy 13						
MSAFP MoM	—	—	1.19	—	—	—
MSAFP <0.5 MoM	1/3 (33%)	—	0/5 (0%)	—	—	—
MSAFP control <0.5 MoM	5.0%	—	7.0%	—	—	—
AFAFP	—	—	1.07 (MoX)	—	—	—
All autosomal trisomies						
MSAFP MoM	—	—	0.8	—	—	—
MSAFP <0.5 MoM	11/41 (26.9%)	—	12/61 (19.7%)	—	—	—
MSAFP control <0.5 MoM	5.0%	—	7.0%	—	—	—
AFAFP	Normal	—	0.81 (MoX)	—	—	—

MSAFP = Maternal serum  $\alpha$ -fetoprotein; MoM = multiples of the median; AFAFP = amniotic fluid  $\alpha$ -fetoprotein; MoX = multiples of the mean.

$\alpha$ -fetoprotein production from an abnormal fetus with immature organs (for instance, the liver) appears to be the most reasonable. We did not find evidence of a similar trend in amniotic fluid values with trisomies 18 and 13; however, numbers are small.

In projecting the potential use of maternal serum  $\alpha$ -fetoprotein screening for fetal Down syndrome and other trisomies, we based our initial calculations on a cutoff point of 0.5 multiples of the median and a figure of 7% for the percentage of our normal prenatal population with values below this point. We subsequently

revised this figure downward to 5% based on the findings of Cuckle and Wald<sup>2</sup> regarding downward revision by ultrasound correction for gestational age. This has also been our experience with our own maternal serum  $\alpha$ -fetoprotein screening project. Based on our study, if all women under age 35 who had maternal serum  $\alpha$ -fetoprotein values less than 0.5 multiples of the median (5%) were offered amniocentesis, 20% of fetal autosomal trisomies would be detected. Calculations were based on Hook's<sup>6</sup> tables regarding frequency of autosomal trisomies and Ontario statistics regarding

<i>Guibaud et al.</i> <sup>6</sup>	<i>Cowchuk and Rush</i> <sup>7</sup>	<i>Total</i>
0.76 (13)	1.0	—
—	0/40 (0%)	40/223 (17.9%)
—	—	—
Normal	0.8 (MoM)(40)	—
—	—	—
—	—	5/23 (21.7%)
—	—	—
—	—	—
—	—	1/8 (12.5%)
—	—	—
—	—	—
—	1.0	—
—	0/59 (0%)	23/161 (14.5%)
—	—	—
—	0.9 (MoM)(59)	—

births by gestational age (1981).<sup>9</sup> In 1981, 115,318 of 122,187 births in Ontario were to women less than age 35. We calculate that 115 infants with trisomy 13, 18, and 21 would be delivered in this group of women (80% trisomy 21). Twenty percent of these would be detected by maternal serum  $\alpha$ -fetoprotein screening (32), and to detect these, 5766 women (5%) would require amniocentesis. The genetic risk, therefore, for an individual patient with a maternal serum  $\alpha$ -fetoprotein value of  $<0.5$  multiples of the median for trisomies 13, 18, and 21 would be 1:180. This is well within the accepted

genetic risk range for genetic amniocentesis for advanced maternal age. If the original figure of a 7% amniocentesis rate is used, the ratio is still one in 357, again comparable to the genetic risk in a 35-year-old patient.

Our data and those of others suggest that serum  $\alpha$ -fetoprotein screening for fetal autosomal trisomies (mainly Down syndrome) is feasible. Pilot studies should be undertaken to confirm the validity of serum  $\alpha$ -fetoprotein screening in clinical practice. Groups that are already engaged in such screening would be best suited to conduct such studies.<sup>10</sup>

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# Staging laparotomy in early epithelial ovarian carcinoma

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It is well known that ovarian carcinoma may have subclinically metastasized at the time of the initial surgical operation when all tumor seemed to be confined to the ovary. A retrospective review of 650 ovarian carcinoma patients from 1976 to 1984 revealed 25 staging laparotomies for early epithelial ovarian carcinoma. Sixteen patients had invasive epithelial ovarian carcinoma, and nine had borderline ovarian carcinomas. Five patients had the stage of their disease changed whereas 20 remained unchanged. Among the staging laparotomy patients, 50% of cases of ovarian carcinoma with ruptured capsules were upstaged as were 33% with those with ascites. Twenty-five percent of cases with invasive epithelial ovarian carcinoma and 12% with borderline ovarian carcinoma were upstaged by a staging laparotomy. As a result of staging laparotomy, 72% of patients were spared treatment. No patient with disease truly confined to the ovaries showed recurrence in spite of receiving no treatment. All patients with disease apparently confined to the ovaries should undergo a staging laparotomy. Only disease remote from the ovary need be treated. If a staging laparotomy is not done, treatment is recommended for apparent Stage I disease. (AM J OBSTET GYNECOL 1986;154:282-6.)

**Key words:** Ovarian carcinoma, laparotomy, metastasis

Ovarian carcinoma is the third most common female genital tract malignancy but remains the leading cause of death among women with gynecologic cancers. Cases of seventeen thousand women in the United States are newly diagnosed each year, with approximately 1% of all women in the nation dying of ovarian carcinoma.<sup>1</sup> The 5-year survival rates are Stage IA, 65%; Stage IB, 52%; and Stage IC, 52%.<sup>2</sup> Of the patients with early-stage disease, 20% to 50% eventually show evidence of relapse.<sup>3</sup> These figures indicate that at least 20% of patients whose ovarian tumor seems confined to the ovary have in fact microscopic metastatic disease remote from the ovary, and hence do not truly have Stage I disease. These patients may be subject to inadequate therapy, since therapy is based on the stage of the disease. Conversely, many centers treat patients who appear to have Stage I disease with adjuvant therapy for possible microscopic metastasis that may have been missed at the primary operation.

Since staging of ovarian carcinoma remains a surgical procedure, the true stage is a function of adequate and complete sampling of known sites of ovarian metastasis. Microscopic metastasis may be found on the pelvic peritoneal surfaces,<sup>4</sup> abdominal peritoneal surfaces,<sup>5</sup> diaphragm,<sup>6</sup> omentum,<sup>7</sup> ascitic fluid,<sup>8-10</sup> and lymph nodes, both pelvic and para-aortic.<sup>7, 11, 12</sup> Staging procedures must obtain samples from the above sites especially

when the ovarian carcinoma seems confined to the ovary(ies).

The object of this study is to assess the impact and yield of a staging laparotomy in determining the stage of ovarian carcinoma in cases where the tumor appears to be confined only to the ovaries and to analyze whether factors of the ovarian tumor such as its histologic type, grade, size, integrity of its capsule, and presence of ascites may be predictive of a higher stage of disease than suggested by the primary operation.

## Material and methods

A retrospective study was undertaken of all the patients who underwent a staging laparotomy for early ovarian carcinoma from 1976 to 1984 at the Manitoba Cancer Treatment and Research Foundation. All patients with ovarian cancer who were registered with the Foundation during that period of time were reviewed, and those patients with a staging laparotomy for epithelial tumors were analyzed.

The staging laparotomy consisted of peritoneal washings from the paracolic gutters and cul-de-sac or ascitic fluid sampling, followed by a total abdominal hysterectomy, bilateral salpingo-oophorectomy, total omentectomy, multiple peritoneal biopsies (between 30 to 50 biopsies) including diaphragmatic peritoneal surfaces, para-aortic and pelvic lymph node sampling, and surgical inspection of the liver and bowel. In cases in which fertility was a strong consideration and the ovarian tumor appeared to be confined to one ovary, the hysterectomy and contralateral salpingo-oophorectomy was replaced with biopsy of the contralateral ovary.

The staging laparotomy was labeled "complete" if all

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**Table I.** Primary surgical procedures

Procedure	Patients		Patients with suspected malignancy	
	n	%	n	%
Biopsy of ovary	1		0	
Cystectomy	2		0	
Unilateral salpingo-oophorectomy	7		0	
Bilateral salpingo-oophorectomy	2		0	
Total abdominal hysterectomy and unilateral salpingo-oophorectomy	1		0	
Total abdominal hysterectomy and bilateral salpingo-oophorectomy	2		0	
Total abdominal hysterectomy, bilateral salpingo-oophorectomy, and omental biopsy and washings	4	16	4	16
Staging laparotomy	6	24	6	24
Total	25	100	10	40

**Table II.** Histology of patients undergoing staging laparotomy

Histologic type	Patients	
	n	%
Serous	5	20
Mucinous	8	32
Endometrioid	9	36
Clear cell	1	4
Mixed	2	8

**Table III.** Ovarian histologic grade of patients undergoing staging laparotomy

Grade	Patients		Histologic distribution (no.)
	n	%	
0-borderline	9	36	Mucinous (5) Serous (3) Mixed (1)
1	11	44	Endometrioid (6) Mucinous (3) Serous (1) Mixed (1)
2	3	12	Endometrioid (2) Serous (1)
3	1	4	Endometrioid (1)
Not applicable	1	4	Clear cell (1)

the above steps were carried out and "incomplete" if lymph node sampling was not done. Patients who had only a total abdominal hysterectomy and bilateral salpingo-oophorectomy with an omental biopsy and washings were not considered to have had a staging laparotomy when the tumor seemed confined to the ovaries. A staging laparotomy was identified as positive if the ovarian tumor stage was changed after the above described laparotomy and as negative if no such change occurred.

International Federation of Gynecology and Obstetrics staging for ovarian carcinoma was followed.

**Table IV.** Apparent initial stages of patients undergoing staging laparotomy

Stage	Patients	
	n	%
IA	18	72
IA1	12	48
IA2	6	24
IB	3	12
IB1	1	4
IB2	2	8
IC	3	12
IIB	1	4

**Table V.** Time interval between primary surgical procedure and staging laparotomy

Interval (mo)	Patients	
	n	%
During primary operation	6	24
<1	5	20
1-2	7	28
2-3	3	12
>3	4	16

In the study group the age distribution, nature of the primary operation, and histologic appearance, grade, and size of the ovarian tumor were reviewed. Patients who had a positive staging laparotomy were then analyzed with respect to the histologic type of the tumor, grade, size, integrity of the capsules, and presence or absence of free tumor cells in the peritoneal cavity. Treatment and survival to date were then analyzed in both the positive and negative groups.

## Results

The cases of 650 patients with ovarian cancer registered with the Manitoba Cancer Foundation between 1976 and 1984 were reviewed. Twenty-five patients had



**Table VI.** Changes in stage of the ovarian tumor

Initial stage	Final stage								No. of patients with positive staging laparotomy
	IA1	IA2	IB1	IB2	IC	IIA	IIB	IIC	
IA1	12								0/12
IA2		3			1	1		1	3/6
IB1			1						0/1
IB2				1				1	1/2
IC					2		1		1/3
IIA						—			—
IIB							1		0/1
Total	12	3	1	1	3	1	2	2	5/25

**Table VII.** Stages of patients who received chemotherapy

Stage	No. of patients
IB2	1
IC	1
IIA	1
IIB	2
IIC	2
Total	7

a staging laparotomy for what appeared to be epithelial ovarian carcinoma localized to the ovaries. Twenty patients (80%) had the surgery after 1981.

Twenty patients had a complete staging laparotomy as described above, whereas in five patients no lymph node sampling was carried out. These patients were operated on before 1979, a time when it was thought that nodal sampling may interfere with the yield of a second-look operation that these patients may be subjected to after a course of chemotherapy.

The age of the patients ranged from 16 to 79 years, with 6 patients (24%) being between 40 to 50 years of age. Thirteen patients (52%) were premenopausal. The primary surgical procedures that these patients underwent are shown in Table I.

Ovarian malignancy was not suspected in 15 patients (60%). Only six patients were subjected to a full staging laparotomy at the time of the initial operation, whereas 19 patients (76%) were reexplored.

The histologic findings for the ovarian tumors of the patients who had staging laparotomies is presented in Table II.

The grade of the ovarian tumor in patients who had staging laparotomies is presented in Table III. Grading was not applicable in one patient with clear cell carcinoma. In 23 patients (92%) the ovary was more than 6 cm in diameter.

The stage of the ovarian tumor before performance of a staging laparotomy is presented in Table IV. One patient, considered to be at Stage IIB, underwent a full staging laparotomy because of concomitant pelvic in-

flammatory disease with adhesions, which shed doubt on whether the tumor was truly Stage IIB.

Nineteen patients (76%) underwent staging laparotomy within 2 months of their initial operation, whereas six patients (24%) had a primary staging laparotomy. No patient was reexplored beyond 4 months of the initial operation. The detailed time interval between the operation and the staging laparotomy is presented in Table V.

The original stage of the ovarian tumor was changed because of the staging laparotomy results in five patients (20%) but remained unchanged in 20. One patient, however, had no lymph node sampling during the staging laparotomy (incomplete staging laparotomy) and was treated for Stage IC disease and subsequently at second-look laparotomy was shown to have para-aortic lymph node disease. If this patient had lymph node sampling during the staging laparotomy, she may have been upstaged beyond Stage IC. Table VI shows the changes in stages on staging laparotomy.

Four patients with a positive staging laparotomy had endometrial carcinoma (80%), and one patient had serous carcinoma (20%). Similarly, one of five patients with serous carcinoma (20%) and four of nine patients with endometrial carcinoma (44%) had a positive staging laparotomy.

One of nine patients with borderline tumors (12%) had a positive staging laparotomy, and three of 11 patients with well-differentiated tumors were upstaged (27%). One of three patients with moderately differentiated tumors (33%) had a positive staging laparotomy. When cases of true invasive disease were separated from those with borderline tumors, 25% had positive staging laparotomy (four of 16).

All patients confirmed to be in Stages IA, IB, or IC (with negative cytologic findings) did not receive any further treatment beyond the staging laparotomy except for two patients who had incomplete staging procedures (no lymph node sampling), one in Stage IC, and one in Stage IB2 with a ruptured capsule. The stages of the patients who had chemotherapy treatment are presented in Table VII.

The follow-up period for most patients (72%) had

**Table VIII.** Present status of the study group according to the results of the staging laparotomy

<i>Staging laparotomy result</i>	<i>Treatment category</i>	<i>Alive without disease</i>	<i>Alive with disease</i>	<i>Dead with disease</i>	<i>Dead without disease</i>	<i>Total</i>
Negative	Not treated	17	0	0	0	17
	Treated (Stages IB2, IC, IIB)	0	0	1	2	3
Positive	Not treated (Stage IC)	1	0	0	0	1
	Treated	2	2	0	0	4
Total		20	2	1	2	25

been <4 years. However, the status of these patients to date is presented in Table VIII.

Of the 23 patients in Stage I, one patient died of a bronchogenic carcinoma and one died of disease after positive nodal sampling on a second-look laparotomy. This patient did not have nodal sampling during the staging laparotomy and died of disease 3 years later. Aside from this one case, none of those confirmed to be in Stage I showed any clinical or radiologic evidence of disease during the follow-up period. The last fatality was the patient with Stage IIB disease who died of an astrocytoma.

Of the five patients who had a positive staging laparotomy, four received treatment with chemotherapy. Two had a negative second-look laparotomy and are alive without disease, while two are still receiving chemotherapy and considered to be alive with disease.

#### Comment

Compared to other gynecologic malignancies, ovarian carcinoma is still plagued by a poor 5-year survival rate even in early stages. These disappointing survival rates are partly due to the nature of the disease and limitations in treatment modalities but are also due to incorrect staging. What may appear to be well-localized disease may in fact have already metastasized along known routes of spread for ovarian malignancies. The fact that an apparent Stage I ovarian tumor may prove to have spread beyond the ovaries at the time of the initial operation has been stressed throughout the literature.

In 1940 Pemberton advocated use of an omentectomy during an ovarian cancer operation, for he believed the omentum might be the source of recurrence. Thirty-five years later, a prospective study showed a 4.7% incidence of omental metastasis in so-called Stage I ovarian carcinoma.<sup>7</sup>

In 1956 peritoneal cytologic studies were shown to reveal early ovarian carcinoma in the absence of clinical ascites.<sup>8</sup> An updated review by the same author in 1974 showed positive cytologic findings in 36% of Stage IA ovarian carcinoma,<sup>9</sup> and another report showed that peritoneal washings changed the stage in 12% of patients.<sup>10</sup> Prospective studies demonstrated that para-aortic lymph nodes are positive in 12% of Stage I pa-

tients<sup>7</sup> and 7% of Stage IA,<sup>12</sup> while pelvic lymph nodes were positive in 8% of Stage I patients.<sup>11</sup> The diaphragmatic peritoneum, with the rich supply of lymphatic channels draining the peritoneal cavity, may harbor tumor emboli. In one series it was determined that 40% of patients with Stages I and II carcinoma had diaphragmatic microscopic disease and 12.5% of patients had metastasis on the anterior peritoneum of the abdominal wall.<sup>5</sup>

Thus sampling these known areas of tumor spread is essential for proper staging of ovarian carcinoma.

In this study 20% of patients who would have been labeled as having Stage I ovarian carcinoma proved to be beyond Stage I, four showing pelvic extension while three showed tumor cells floating freely within the peritoneal cavity. If one adds the patient who did not have lymph node sampling at staging laparotomy but proved to have such involvement on second-look laparotomy, then this percentage would increase to 24%. This is comparable to the figures from other studies with percentages of 31%<sup>13</sup> and 25%.<sup>14</sup>

If one considers the histologic findings of the tumor, 80% of those with a positive staging laparotomy had endometrioid carcinoma and 44% of all those patients with endometrioid carcinoma had a positive staging laparotomy. There was no comment on the epithelial histologic types of ovarian tumors upstaged in the study by Young et al.,<sup>13</sup> but undifferentiated adenocarcinoma was prominent in the study by Free et al.<sup>14</sup> No patients in this series had undifferentiated adenocarcinoma, but then it would seem surprising to have such histologic findings in apparent Stage I disease.

It was obvious that the poorer the differentiation, the higher the chances for a positive staging laparotomy in Stage I disease. Thus while 12% of the borderline tumors were upstaged, 33% of the moderately differentiated tumors were upstaged. It is interesting to note that the one case with a poorly differentiated tumor did not have a positive staging laparotomy.

Integrity of the capsule proved critical in preventing microscopic tumor spillage. Webb et al.<sup>15</sup> stressed that patients with ruptured capsules had a progressive decrease in survival rates in Stage I disease.

In our study four of eight patients with ruptured capsules (Stages IA2 and IB2) and one of three patients

with positive peritoneal washings (Stage IC) were upstaged.

All the patients with positive staging laparotomy proved to have their microscopic metastatic disease in the pelvis. No patient was upstaged to Stages III or IV. Only one patient who had no lymph node sampling during staging laparotomy proved to have para-aortic nodal disease on a second-look laparotomy. If lymph node sampling was done at the staging laparotomy, it is likely that the lymph node may have been positive and hence conforming to the incidence of lymph node involvement in Stage I disease reported in the literature.<sup>7,12</sup> Larger numbers of Stage I disease are required to confirm the literature incidences of microscopic disease in the omentum, diaphragmatic surfaces, and lymph nodes.

Thus from this review it would appear that patients are at a higher risk of not truly having Stage I disease if they harbor a less well-differentiated endometrioid carcinoma, especially if this is compounded by a tumor >5 cm in diameter with a ruptured capsule and positive peritoneal washings.

Perhaps the greatest use a staging laparotomy serves is as a guide to subsequent treatment. If disease is truly limited to the ovaries (Stage I), which have been removed, then no further treatment is needed. Withholding treatment, however, in those patients with apparent Stage I disease who have not undergone a complete staging laparotomy may result in up to a 40% recurrence rate as demonstrated by the approximate 60% survivals for untreated Stage I ovarian carcinoma. Conversely, the use of adjuvant treatment, either abdominal radiotherapy<sup>16</sup> or chemotherapy, for true Stage I disease is not indicated. By ruling out microscopic disease beyond the ovaries by a staging laparotomy, such treatments can be avoided. In our study, as a result of staging laparotomies, 72% of the patients were spared having to undergo further treatment.

In cases in which the initial surgical procedure shows an ovarian tumor to be in Stage IA1, sampling the contralateral ovary is indicated to rule out bilaterality and to confirm the integrity of the contralateral ovarian capsule. Whether a complete staging laparotomy is indicated can only be assessed after obtaining a larger number of patients with apparent Stage IA1 disease in the future. Until then, however, we still recommend a complete staging laparotomy even for tumors localized to ovaries with apparent intact capsules. In this limited series, none of our Stage IA1 patients were upstaged.

The false negativity of a staging laparotomy in ovarian cancer remains to be determined. To date no pa-

tient with Stage I disease confirmed by a staging laparotomy has shown any recurrence of their disease in spite of not receiving treatment. A longer period of follow-up, however, is indicated to assess whether any of these patients will demonstrate a recurrence in the years to come.

In conclusion, a staging laparotomy is essential before any definitive treatment in apparent Stage I ovarian carcinoma. Only disease remote from the ovaries need be treated. If a staging laparotomy is not carried out, then treatment would still be recommended even for apparent Stage I disease, since a significant number of these patients will actually have more advanced disease.

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# CA 125 surveillance and second-look laparotomy in ovarian carcinoma

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CA 125 was evaluated as a tumor marker in 31 patients undergoing treatment for ovarian carcinoma, 17 of whom had second-look laparotomies. At the time of second-look laparotomy, 14 patients had CA 125 values in the normal range. Six of these patients had a positive second-look laparotomy. Although normal CA 125 values do not obviate the need for second-look laparotomy in treatment planning, rising or falling trends reflected clinical disease progression or regression in 80% of the cases. (*AM J OBSTET GYNECOL* 1986;154:287-9.)

**Key words:** Ovarian cancer, CA 125 surveillance, second-look laparotomy, tumor marker

The clinical evaluation of ovarian carcinoma tumor burden during treatment and follow-up is difficult. Current treatment involves surgical reevaluation for tumor status after a course of chemotherapy or radiotherapy.<sup>1,2</sup> Identification of a sensitive tumor marker may one day preclude the necessity for second-look laparotomy.

CA 125 is a high-molecular weight glycoprotein found in embryonal coelomic epithelium. This antigen was identified by a murine monoclonal antibody, after immunizing mice with cultured epithelial ovarian cancer cells.<sup>1</sup>

CA 125 has been identified with serous, endometrial, clear cell, and undifferentiated ovarian tumors, and the antibody does not bind to normal adult ovarian tissue. A radioimmunoassay has been developed to detect CA 125 in serum.<sup>3</sup>

To assess whether CA 125 levels predict disease status at second-look laparotomy, serum levels were measured in a group of patients presenting with nonmucinous epithelial ovarian carcinoma. Sequential levels were also assessed in a group of patients whose clinical progression of disease precluded second-look laparotomy.

## Material and methods

A radioimmunoassay with use of polystyrene beads coated with monoclonal (IgG) OC 125 was developed by Centocor to detect CA 125 in 100  $\mu$ l portions of serum.<sup>3</sup> By simultaneous incubation with iodine 125—

labeled OC 125 and comparison to a serially diluted antigen preparation used as a primary reference standard, CA 125 levels are expressed in units per milliliter.

During a 1-year period, 31 patients with nonmucinous epithelial ovarian carcinoma presenting to the London Regional Cancer Centre, London, Canada, were studied. Patients' ages ranged from 33 to 76 years (mean, 54.5 years). Surgical staging (International Federation of Gynecology & Obstetrics) was assigned at initial assessment. Three cases were Stage I, two were Stage II, 22 were Stage III, and four were Stage IV. The morphologic study of the tumors included 14 serous, five endometrioid, two clear cell, and 10 undifferentiated.

All patients undergoing second-look laparotomy were evaluated by one of us (H. H. A.). Intraperitoneal washings were taken on entering the abdomen, and after exploration of all peritoneal surfaces, any gross tumor nodules were removed when indicated. In the presence of no detectable disease, multiple biopsy specimens (numbering 30 to 50 in total) were taken from the pelvic floor and sidewalls, paracolic gutters, mesentery of small and large bowel, and diaphragms. Retroperitoneal node sampling was carried out if not performed at the initial operation. A total of 158 separate serum CA 125 values were determined at various times during the course of treatment of 31 patients. CA 125 levels were made available and analyzed retrospectively, having been unknown to us at the time of second-look laparotomy. Patients with Stage IV disease and those with clinically obvious rapid tumor recurrence and progression were not subjected to second-look laparotomy. In addition, at the time of evaluation of these data, a number of patients being treated with chemotherapy were being followed clinically while awaiting surgical reevaluation in the near future.

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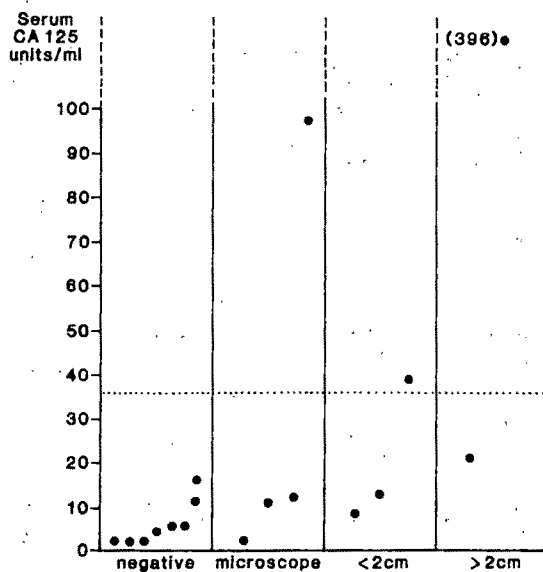


Fig. 1. Preoperative CA 125 level and findings at second-look laparotomy. Size refers to greatest diameter of tumor nodules present. Dotted line indicates upper limit of normal range.

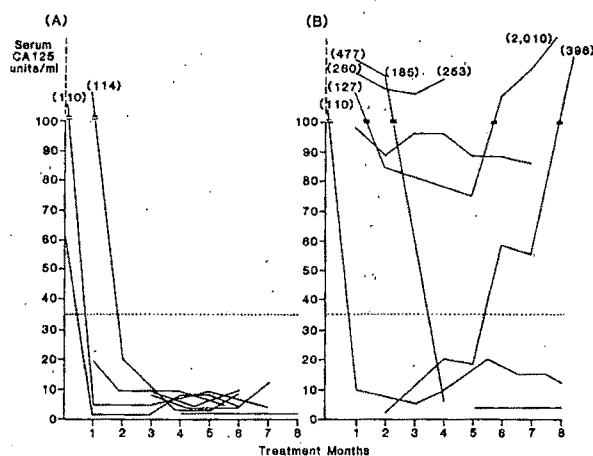


Fig. 2. A, Serial CA 125 levels resulting in negative second-look laparotomy. B, Serial CA 125 levels resulting in positive second-look laparotomy or biopsy proven recurrence. Dotted line indicates upper limit of normal range.

## Results

Of the 31 patients studied with CA 125, 17 underwent second-look laparotomy. Although 16 of 17 patients were clinically free of disease before surgical re-evaluation, nine patients (53%) were found to have tumor present at second-look laparotomy.

Preoperative CA 125 values were within the normal range ( $<35$  U/ml) in 14 patients. However, six of these 14 patients (43%) had a positive second-look laparotomy (Fig. 1). Of these, three had microscopic disease only, two had multiple nodules of  $<2$  cm in greatest diameter, and one had bulk disease ( $>2$  cm in diameter). In a comparison of second-look laparotomy find-

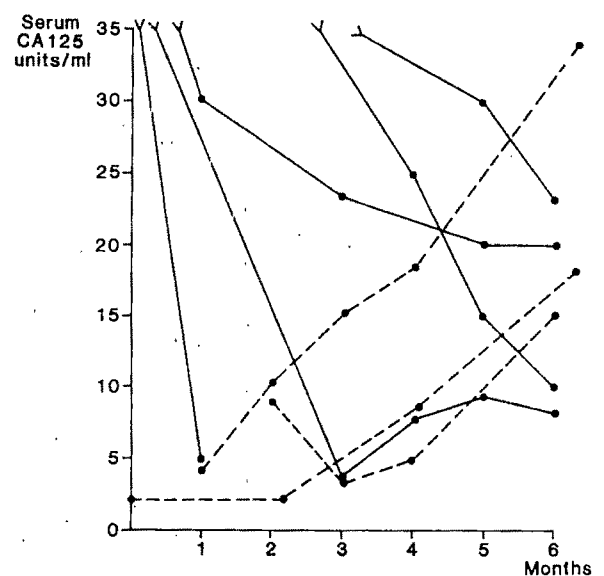


Fig. 3. Serial CA 125 levels within the normal range and clinical follow-up. Dashed line, clinical progression; solid line, clinical regression.

ings with preoperative CA 125 levels, with use of the standard 35 U/ml as the accepted upper limit of normal, the sensitivity was 33%, the specificity 100%, the positive predictive value 100%, the negative predictive value 57%, and the overall accuracy 65%.

Of the original 31 patients, 22 have been evaluated with a minimum of three serial CA 125 determinations during the course of their treatment and follow-up. These patients' values have been plotted individually, with seven cases having a negative second-look laparotomy shown in Fig. 2, A, seven having a positive second-look laparotomy or biopsy-proven recurrence shown in Fig. 2, B, and eight receiving only clinical follow-up. Patients with less than three CA 125 determinations during the course of treatment were not included. No patient with a negative second-look laparotomy maintained an elevated serum CA 125 value at the time of surgery (Fig. 2, A). Two patients with a positive second-look laparotomy (both with multiple nodules of  $<2$  cm diameter) showed initially elevated CA 125 values that fell into the normal range (Fig. 2, B). One patient had a persistently low CA 125 during her disease course and had a positive second-look laparotomy (Fig. 2, B).

## Comment

A reference value for a normal  $<35$  U/ml was initially established by Klug et al.<sup>5</sup> to include 56 normal healthy individuals and to exclude 86 of 105 (82%) of ovarian cancer patients. These investigators have subsequently shown that only 1% of 888 apparently healthy persons have a level of  $\geq 35$  U/ml, whereas 83 of 101 (82%) with surgically proved ovarian carcinoma had elevated

levels. Bast et al.<sup>6</sup> have also shown that levels correlate with the clinical course of disease in 42 of 45 instances (93%), by means of a doubling or halving of serum value to reflect disease progression or regression.<sup>6</sup>

In our series, disease progression or regression was reflected in a doubling or halving of serum CA 125 levels in 16 of 20 patients (80%).

It is interesting that clinical regression has correlated well with CA 125 values falling from above the established normal, and continuing to fall within the normal range in five patients (Fig. 3). However, although still within the <35 U/ml range, three of eight (38%) patients followed clinically have been identified to have tumor progression. All three of these patients had a large tumor burden that was clinically obvious and increasing in extent. The clinical evaluation of progressive tumor burden was reflected in rising serial CA 125 values although all values were below 35 U/ml (Fig. 3). An attempt to demonstrate trends within the normal range among the patients undergoing second-look laparotomy with clinically undetectable disease failed to reveal a similar pattern. However, if rising CA 125 levels are noted even within the normal range, we remain highly suspicious of disease progression or recurrence.

Although this tumor marker has proved itself to be superior to any marker previously identified for epithelial ovarian carcinomas, the practical significance remains questionable, since 43% of patients who underwent second-look laparotomy with normal CA 125 values had tumor present. Expression of a cell surface antigen such as CA 125 may be dependent on cellular differentiation as well as gross tumor volume, and such expression may be further altered by chemotherapy.

Although not as accurate at predicting biologic tumor behavior as  $\beta$ -human chorionic gonadotropin is for gestational trophoblastic disease, sequential CA 125 determinations in the follow-up of patients with ovarian carcinoma have shown remarkable trends. No patient who had a negative second-look laparotomy had an elevated CA 125 level. Patients with initially elevated CA 125 levels consistently demonstrated a poor outcome if these values failed to fall below 35 U/ml. Those patients with CA 125 levels showing a twofold increase over time although still remaining within the normal range, should be observed closely for disease progression. However, normal CA 125 levels do not obviate the need for second-look laparotomy in ovarian carcinoma treatment planning at this time.

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# Ovarian carcinoma of low malignant potential: Staging and treatment

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A review of 65 cases of ovarian carcinoma of low malignant potential registered at the Manitoba Cancer Treatment and Research Foundation over a 7½-year period was undertaken. Eighty-four percent of patients presented with Stage I disease, which was confirmed by primary staging laparotomy in 25%. The average age at diagnosis was younger than that commonly found in patients with invasive carcinoma. Fourteen patients received postoperative chemotherapy, of whom 10 were evaluated with second-look laparotomy. No patient with macroscopic residual disease after initial surgery was cured by chemotherapy. This report emphasizes the need for a prospective controlled study to evaluate adjunctive chemotherapy in the treatment of these tumors. (AM J OBSTET GYNECOL 1986;154:290-3.)

**Key words:** Ovarian cancer, low malignant potential, staging, treatment

Epithelial ovarian carcinomas of low malignant potential have been studied with increasing interest over the past decade. The clinical entity was initially reported by Taylor<sup>1</sup> in 1929, and a pathologic definition was accepted by the International Federation of Gynecology and Obstetrics (FIGO) in 1971<sup>2</sup> and by the World Health Organization in 1973.<sup>3</sup> However, the diagnosis is still associated with uncertainty with respect to both optimum surgical management and the need for postoperative therapy.

Pathologically these tumors have been identified as intermediate between cystadenomas and cystadenocarcinomas.<sup>4</sup> Their clinical course is relatively benign in comparison to that of the invasive carcinomas. Stage I tumors have an excellent prognosis.<sup>5</sup> Those tumors greater than Stage I demonstrate an indolent behavior with good survival rates, although studies with long-term follow-up illustrate they do have the capacity to metastasize and can be fatal.<sup>6</sup>

Epithelial carcinomas of low malignant potential comprise between 9.2%<sup>6</sup> and 20%<sup>7</sup> of ovarian malignancies, and patients with these tumors tend to present at an earlier stage of disease than those with invasive carcinomas. The average age of patients at diagnosis is younger than in those with invasive carcinoma.<sup>5</sup>

Treatment has ranged from conservative surgical procedures to aggressive tumor-debulking in combination with adjuvant cytotoxic chemotherapy and/or

radiotherapy. However, because of the good prognosis associated with these tumors, it has been difficult to evaluate the results of these various modes of treatment. This review was undertaken to study the clinical characteristics of patients with epithelial tumors of low malignant potential and to assess the value of staging procedures and subsequent cytotoxic chemotherapy on the clinical course.

## Material and methods

A retrospective review of the charts of 688 patients with ovarian carcinoma registered at the Manitoba Cancer Treatment and Research Foundation from July 1, 1976, to December 31, 1984, was conducted. The records of 65 patients with a diagnosis of ovarian carcinoma of low malignant potential as defined by World Health Organization criteria<sup>3</sup> were selected for further study. All pathologic findings were confirmed by tissue review in the Department of Pathology either at Health Sciences Centre, Winnipeg, or at St. Boniface General Hospital, Winnipeg. All cases of epithelial ovarian tumors of low malignant potential were included.

## Results

The cases were evenly distributed over the study period and represented 9.4% of all patients with ovarian malignancies presenting during that time. The age at diagnosis ranged from 17 to 91 years. Thirty-five patients (53.8%) were <50 years of age at diagnosis.

The most common presenting symptoms were lower abdominal discomfort (28.1%) and increasing abdominal girth (20.3%), although 17.1% were asymptomatic, with a mass being detected incidentally on routine examination.

Surgical management was reviewed. Sixteen patients (25%) had a unilateral salpingo-oophorectomy only.

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**Table I.** Distribution of histologic findings by stage of patients

Stage	Histologic findings		Total
	Mucinous	Serous	
IA	32	14	46
IB	—	2	2
IC	2	3	5
IIA	—	1	1
IIB	—	2	2
IIC	1*	1*	1*
III	2	5	7
IV	—	1	1
	37	29	55

\*One patient had mixed mucinous and serous elements, both of low malignant potential.

Forty-two (65%) had a total abdominal hysterectomy and bilateral salpingo-oophorectomy. Eleven patients (17%) had an omental biopsy or omentectomy and/or peritoneal washings for cytology as part of the initial surgical procedure. Fifteen patients (23%) underwent a primary staging laparotomy including peritoneal cytology, omentectomy, pelvic and precaval lymph node sampling, and multiple peritoneal biopsies.

Fifty-five patients (84.6%) had Stage I disease. This was confirmed by primary staging laparotomy in 14 of these patients (25%). Two (3%) had Stage II disease, seven (10.7%) had Stage III disease, and one (1.5%) had Stage IV disease on the basis of a malignant pleural effusion. One case was upgraded from Stage I to Stage III as a result of a staging laparotomy. Two were downgraded from Stage II to Stage I. In both the latter cases the stage had been assigned initially on the basis of adhesions in the pelvis requiring sharp dissection, and subsequently primary staging laparotomy was negative.

Five patients (7.6%) had cell-positive ascites at laparotomy. Twenty-three (35.3%) had peritoneal cytology performed, of which specimens four (17.3%) were positive for malignant cells. Thirty-four patients (52.3%) had an omentectomy or omental biopsies of which six (17.6%) were positive. Two of these six cases were subsequently upgraded from Stage I to Stage III on the basis of this microscopically positive omental biopsy in the absence of any other disease.

Details of histologic findings according to stage are illustrated in Table I.

Postoperative treatment is detailed in Table II according to stage. No patient received radiotherapy. The 14 patients in whom a primary staging laparotomy confirmed Stage I disease were not treated. The five patients who were treated with Alkeran were all diagnosed as having Stage I disease but with inadequate staging procedures. Rather than subject the patients to another laparotomy, a decision was made in conjunction with their referring physicians to treat them with Alkeran.

**Table II.** Postoperative treatment according to stage

Stage	No. of patients	Treatment
I	8	Unknown
	42	No treatment
	5	Alkeran
II	2	Alkeran
III	1	No treatment
	2	Alkeran
	3	Adriamycin/cisplatin
	1	Adriamycin/Alkeran
IV	1	Hexamethylmelamine
		Adriamycin/cisplatin

Three of these patients subsequently had a second-look laparotomy, of which one was microscopically positive. Ten patients (five, Stage I; one, stage II; four, stage III) who had no evidence of disease clinically following chemotherapy underwent a second-look laparotomy. Of these procedures, five were positive and five negative. Details are included in Table III. All patients with disease greater than Stage I with macroscopic disease remaining after initial laparotomy had positive second-look laparotomies after treatment.

Two cases were upgraded on the basis of a single positive omental biopsy. One patient was treated with 12 courses of Alkeran (1 mg/kg over 5 days) at monthly intervals and subsequently had a negative second-look laparotomy. The second patient, treated on the basis of a single microscopic focus of tumor in her omentum, also had a negative second-look laparotomy after treatment with hexamethylmelamine, Adriamycin and Alkeran for 13 courses. No patient with a negative second-look laparotomy has had a recurrence of disease.

One patient with Stage III disease was not treated. Peritoneal studding was diagnosed pathologically as endosalpingiosis, and the patient refused further treatment. She has been followed now for 60 months, remaining asymptomatic with no clinical evidence of disease.

Follow-up in this study is short and ranges from 2 to 90 months. Present status of patients is documented in Table IV. It is of note that 21 patients were lost to follow-up, all of whom had Stage IA disease. However, in none of these patients was the stage confirmed by primary staging laparotomy. These patients were not evaluated by the gynecologic oncology service, many of them being diagnosed and treated in other parts of the province. However, their charts were sufficiently complete for this retrospective review. As long as they have remained in Manitoba, none have died of disease. No patient with disease greater than Stage I has been lost to follow-up.

Of the six patients alive with disease, one is presently



**Table III.** Ovarian carcinoma of low malignant potential with second-look laparotomy

Patient No.	Stage	Disease status after initial laparotomy	Disease status after second-look laparotomy
1	IA	Negative	Positive
2	IA	Negative	Positive
3	IA	Negative	Negative
4	IB	Negative	Negative
5	IC	Negative	Negative
6	IIC	Microscopic	Positive
7	III	Microscopic (1 biopsy positive)	Negative
8	III	Microscopic (1 biopsy positive)	Negative
9	III	Macroscopic (<2 cm)	Positive
10	III	Macroscopic (<2 cm)	Positive

**Table IV.** Staging and present status in 65 cases of ovarian carcinoma of low malignant potential

Stage	No. of cases	No evidence of disease	Alive with disease	Death from disease	Death from other cause	Lost to follow-up
I	55	28	—	—	6	21
II	2	—	2	—	—	—
III	7	3	4	—	—	—
IV	1	—	—	1	—	—
Total	65	31	6	1	6	21

**Table V.** Completed months of follow-up of known survivors

Completed follow-up (mo)	No. of patients
<12	2
12	6
24	8
36	6
48	2
60	3
>60	17

undergoing chemotherapy and will be eligible for a second-look laparotomy within 6 months. The other five are alive and well, their disease status having been assigned on the basis of a positive second-look laparotomy in three patients and by clinical examination in two. Three of the five have been followed for >60 months, one for 52 months, and one for 15 months. The length of survival is tabulated in Table V.

#### Comment

The proposal of the International Federation of Gynecology and Obstetrics in 1961 that epithelial tumors of the ovary be divided into three categories (benign, malignant, and intermediate) was followed by acceptance of the classification for usage in 1971. The intermediate group consists of cystadenomas with proliferative activity of epithelial cells and nuclear abnormalities but absence of infiltrating destructive growth.<sup>8</sup> The World Health Organization recognized this classification and has referred to these tumors as carcinomas of low malignant potential or tumors of bor-

derline malignancy.<sup>3</sup> Several authors have suggested that the latter terminology be abandoned in favor of the former to emphasize the capacity of these neoplasms to cause death.<sup>9,10</sup>

Barnhill et al.<sup>11</sup> reported the histologic findings of 82% of 94 tumors of low malignant potential as being serous and 18% mucinous. Aure et al.<sup>7</sup> found 50% of 64 tumors to be mucinous and 46% serous, and these findings are supported in this study. The difference in reported percentages may reflect different distribution by stage, since the majority of early-stage tumors in this study were mucinous. Pathologic evaluation becomes extremely important in making a diagnosis, particularly of large mucinous lesions in which there can be considerable variation from one area of tumor to another. Hart and Norris<sup>12</sup> have recommended that one tissue block be taken for pathologic evaluation for each 1 to 2 cm of the tumor's maximum diameter.

It has been clearly established in several series that these tumors occur in a younger population of women than in those who develop invasive epithelial carcinomas.<sup>3,7,13</sup> The findings in this study concur. Eighty-one percent of tumors in this study were Stage I at diagnosis, which is similar to the result obtained by Aure et al.<sup>7</sup> although two series have reported only 53%<sup>3</sup> and 50%<sup>11</sup> of patients with Stage I disease. Confirmation of stage by primary staging laparotomy has not been standard procedure in the literature. In this study 14 of 55 Stage I cases (25%) were confirmed by primary staging laparotomy. Several authors have advocated less radical surgery for this group of patients, particularly if retention of child-bearing capacity is an issue.<sup>14,15</sup> Unilateral salpingo-oophorectomy is adequate therapy if a

thorough staging procedure confirms Stage IA disease.<sup>5,14</sup> Two of five cases staged as IA without primary staging laparotomy subsequently had positive second-look laparotomies emphasizing the need for adequate staging.

Sixteen percent to 18% of borderline tumors are Stage II or greater.<sup>4,7</sup> The majority of advanced cases are serous in origin. Debulking of all gross tumor is advocated in more extensive disease.<sup>3</sup> Extra ovarian implants may arise by metastases or by in situ development from coelomic epithelium.<sup>1</sup> These implants rarely infiltrate deeply, and it may be possible to incise the peritoneum around a tumor nodule, thus removing it completely. Mortality from tumors of low malignant potential occurs almost exclusively in the presence of peritoneal implants. Russell<sup>4</sup> has suggested that there may be a subgroup of patients with Stage II disease whose implants show local invasion and whose lesions should be reclassified as invasive carcinoma.

Treatment has been variable, and reports of more than a few uniformly treated cases with adequate follow-up are sparse. Julian and Woodruff<sup>5</sup> used radiotherapy in an unspecified number of cases, and Nikrui<sup>6</sup> reported adjuvant radiotherapy in 10 cases. The effectiveness of chemotherapy for advanced disease is unknown, a variety of different regimens having been used over the years.<sup>6</sup> There have been no reports of prospective randomized trials for advanced disease to clarify this issue.<sup>9</sup> These tumors are characterized by indolent growth and may not respond to cytotoxic chemotherapy. Rapidly dividing cells are most affected by cytotoxic agents that act at different stages during the cell cycle to retard or prevent further mitoses. Cells that replicate very slowly are largely protected from the effects of these drugs unless they are in the critical stage when the drug is delivered. Survival rates reported in the literature indicate the good prognosis for patients with this tumor.<sup>7,11,14</sup> The crucial question is whether the various forms of treatment used have any effect on the disease.

Although the number of patients with advanced disease is small and follow-up is relatively short, this study confirms the clinical impression that ovarian tumors of low malignant potential are difficult to treat. It is our clinical impression on the basis of this series that, in view of their excellent prognosis, patients with Stage I epithelial ovarian carcinoma of low malignant potential

confirmed by a primary staging laparotomy should not receive adjunctive therapy. Furthermore, in this series of 65 patients, 14 received chemotherapy and no patient with macroscopic disease at the conclusion of initial surgery had a negative second-look laparotomy after treatment, either with single-agent Alkeran or platinum-containing combination chemotherapy. These findings reiterate the need for a multicenter, prospective, randomized study that includes staging laparotomy, adequate sectioning of specimens, randomized postoperative chemotherapy or radiotherapy, and second-look laparotomy to histologically and surgically assess response. Only after this information is available and long-term follow-up is achieved will there be any consensus on whether further treatment is indicated for advanced stages of this tumor.

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# Quantitative two-dimensional echocardiographic assessment of fetal cardiac growth

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High-resolution, real-time cardiac imaging and Doppler measurements of blood flow have the potential of extending the fetal cardiovascular profile beyond heart rate monitoring alone. The development of normal standards is a prerequisite to the application of these capabilities. To quantify fetal cardiac growth and explore the potential applications, we performed real-time, two-dimensional echocardiography in a cross-sectional study of 75 normal pregnancies from 17 to 40 weeks gestation. Left and right ventricular, left and right atrial, and aortic root measurements were obtained. Regression analysis showed that the best correlation for ventricular and aortic dimensions with gestational age or biparietal diameter was a straight line ( $y = mx + b$ ). Ten normal fetuses were then serially monitored. Cardiac dimensions fell within the confidence range of the regression models, and most cases exhibited similar growth slopes. Finally, five abnormal cases were studied to demonstrate the use of these data in diagnosing altered cardiac structure and function. (AM J OBSTET GYNECOL 1986;154:294-300.)

**Key words:** Fetal cardiac growth; real-time, two-dimensional echocardiography; Doppler

An important prerequisite to the proper application of fetal echocardiography is the development of normal cardiac growth curves. To date, growth has been quantified by comparing M-mode dimensions with gestational age<sup>1,2</sup> and two-dimensional measurements with estimated fetal weight.<sup>3</sup> In this article, we present our data on fetal cardiac growth as a function of gestational age and biparietal diameter with the use of two-dimensional, cross-sectional imaging. In addition, the study illustrates, by example, potential applications of the information derived.

## Material and methods

**Group 1.** Informed consent to carry out fetal echocardiography was obtained from 75 patients with normal pregnancies between 17 and 40 weeks' gestation. All studies were performed with a Hewlett-Packard 77020A real-time phased-array ultrasound imaging system, and measurements were obtained from frozen-frame video-recorded two-dimensional images. The videotapes were reviewed on a frame-by-frame analysis. The frame-to-frame interval was 33⅓ msec. On the basis of a fetal heart rate averaging 120 to 150 bpm (which corresponds with a beat-to-beat interval between

400 and 500 msec), there was an average of 12 to 15 frames per cardiac cycle. The fetal heart was initially scanned to exclude major anomalies, according to a method we have previously described.<sup>1</sup> Cross-sectional views of the apical four-chamber, high parasternal short axis and the parasternal long axis were obtained. Using an on-line electronic caliper system, we obtained transverse dimension measurements of the left and right ventricles, the left and right atria, and the aortic root. The apical four-chamber view was used for all dimensions except the aortic root. The maximal internal transverse dimensions of the left and right ventricles were obtained during diastole at the tips of the atrioventricular valves just prior to closure in a plane perpendicular to the interventricular septum.<sup>3</sup> Atrial dimensions were obtained during ventricular systole, just after atrioventricular valve closure, with the use of the widest visible internal diameter in a plane perpendicular to the interatrial septum. The aortic root was measured by the leading edge method at the level of the aortic valves, during valve closure, with the use of either the short axis or the parasternal long axis. All dimensional data represent an average of three measurements.

Regression models, along with 5% and 95% confidence bands, were then evaluated for the left and right ventricles and the aortic root, as functions of gestational age and biparietal diameter, and for right ventricle versus left ventricle. The regression lines for cardiac dimensions against biparietal diameters have fewer data points, since we excluded 22 biparietal diameter measurements that preceded the echo study and were done outside our unit.

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**Table I.** Range of measurements obtained, SEE, and *r* for various dimensions against biparietal diameter, age, and left ventricular measurement

	<i>Left ventricle</i>	<i>Right ventricle</i>	<i>Left atrium</i>	<i>Right atrium</i>	<i>Aortic root</i>
Range (cm) (17-40 wk)	0.4-1.6	0.4-1.9	0.4-1.6	0.6-1.6	0.24-1.0
SEE	0.17	0.18	0.19	0.15	0.13
<i>r</i> value					
Biparietal diameter	0.84	0.90	0.79	0.85	0.85
Gestational age	0.84	0.86	0.82	0.87	0.74
Left ventricular measurement	—	0.90	0.80	0.87	0.72

**Group 2.** We next evaluated serially, 10 patients with normal pregnancies on two or three occasions, to see how well the cardiac dimensions and growth patterns fit the previously derived regression models.

**Group 3.** Finally, the following five abnormal situations were selected to illustrate how this information might be applied: (1) Rh isoimmunization, (2) possible cardiomegaly noted on ultrasound, (3) fetal tachyarrhythmia, (4) fetal bradycardia, and (5) intrapartum decelerations.

## Results

**Groups 1 and 2.** All neonates in groups 1 and 2 had normal outcomes. Dimensions for the four cardiac chambers and the aortic root exhibited a threefold to fourfold increase in diameter between 17 and 40 weeks' gestation. The ratios of right ventricle/left ventricle (1.16) and right atrium/left atrium (1.12) support the concept of right heart dominance. Regression analysis showed that the best correlation for ventricular and aortic dimensions with gestational age or biparietal diameter was a straight line ( $y = mx + b$ ). There was minimal flare of the 5% and 95% confidence bands for individual values at either end of the regression lines. Table I lists the range of measurements obtained from 17 to 40 weeks, the standard error of the estimate (SEE), and correlation coefficients (*r*) for the various dimensions against biparietal diameter, age, and left ventricle measurement.

Figs. 1, 2, 3, 4, 5, and 6 are the growth curves with 5% and 95% confidence bands for individual predicted values. Fig. 7 is the regression line with confidence bands for the right ventricle as a function of the left ventricle.

Figs. 8 and 9 show that the cardiac dimensions for the 10 serially monitored fetuses fell within the confidence range of the regression models and that most cases exhibited similar growth slopes, with a slightly stronger fit for the left ventricular dimensions (Fig. 8).

## Group 3

**Case 1.** An Rh-isoimmunized fetus had normal cardiac dimensions at 23 and 30 weeks' gestation (Figs. 8 and 9). At 31.5 weeks, fetal movement diminished. Amniocentesis for determination of bilirubin levels was un-

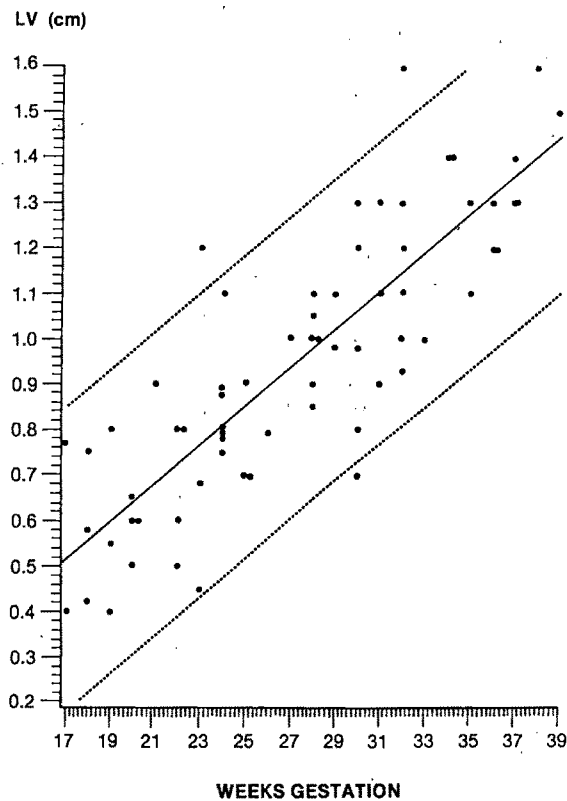
satisfactory, as a large anterior placenta resulted in specimen contamination with hemolyzed red blood cells. Ultrasound suggested "a small amount of fetal ascites." Echocardiography revealed that both the left ventricle and the right ventricle had enlarged acutely during the previous week. A nonstress test that was initially reactive became nonreactive within 24 hours. A hydropic, anemic infant with cardiomegaly was delivered by cesarean section. The condition of the infant improved after several exchange transfusions.

**Case 2.** This patient was referred at 37 weeks with an ultrasound diagnosis of possible fetal cardiomegaly. Echocardiography revealed a huge right atrium measuring 6.1 cm, part of which was an atrialized right ventricle (Fig. 10). An abnormal, apically displaced tricuspid valve was present. Ebstein's anomaly was diagnosed and prostaglandin infusion was started after delivery. Operation on day 16 confirmed the diagnosis. During chest closure, the heart began to fibrillate, and resuscitative attempts failed. Cause of death was right-sided myocardial failure.

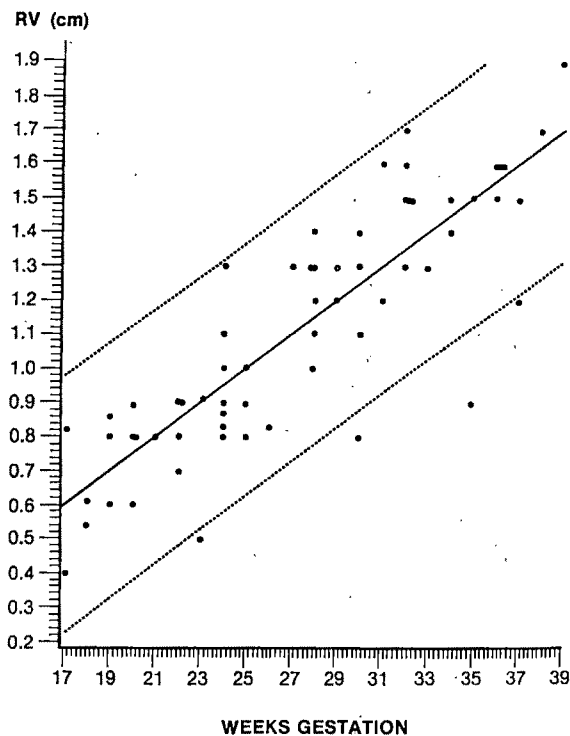
**Case 3.** At 39 weeks, this fetus of a preeclamptic patient was found to have a supraventricular tachyarrhythmia. The two-dimensional echocardiogram revealed right heart enlargement with a right ventricular dimension of 2.2 cm (Fig. 9). Emergency cesarean delivery was performed. The newborn echocardiogram confirmed right atrial and right ventricular enlargement, as well as moderate tricuspid regurgitation. An electrocardiogram revealed right atrial hypertrophy, right ventricular dominance, and T-wave inversion. The neonate was felt to have suffered transient myocardial ischemia related to in utero hypoxia. At an 8-month examination, the child was well.

**Case 4.** A fetal biophysical profile, performed at 37 weeks because of diminished fetal activity, revealed a nonreactive nonstress test. During the ultrasound portion of this test, it was apparent that the true fetal heart rate was 60 to 65 bpm (half the printout rate on the external monitor). An echocardiogram obtained minutes later revealed four-chamber enlargement and a profoundly hypokinetic right ventricle and left ventricle. The urgency of the situation precluded our obtaining accurate chamber measurements and, in fact,

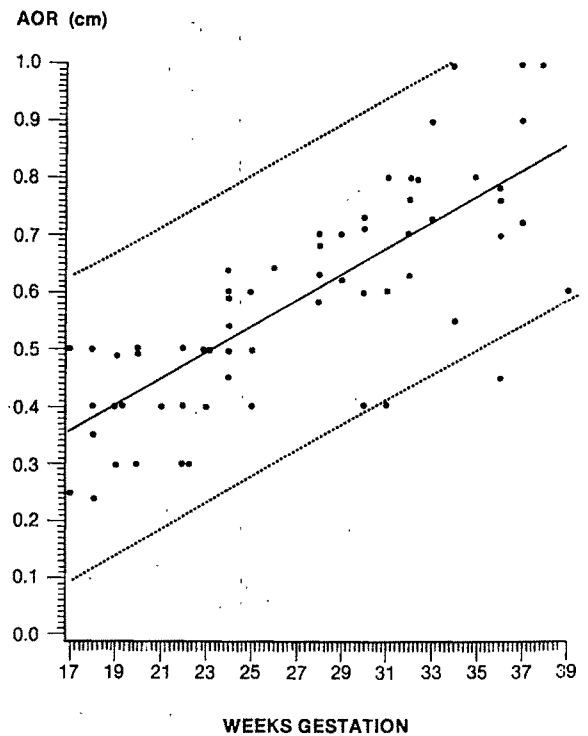




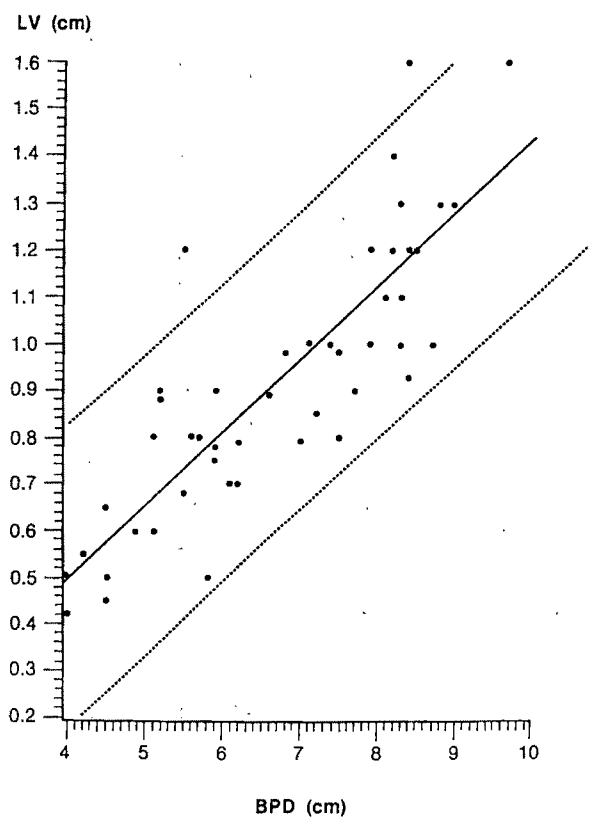
**Fig. 1.** Maximal left ventricular internal dimension (LV) plotted against gestational age. Dotted lines represent the 95% confidence limits of individual predicted values ( $n = 67$ ,  $y = 0.042x - 0.205$ ,  $r = 0.842$ ,  $p = 0.0001$ ).



**Fig. 2.** Maximal right ventricular internal dimension (RV) plotted against gestational age ( $n = 63$ ,  $y = 0.050x - 0.25$ ,  $r = 0.857$ ,  $p = 0.0001$ ).



**Fig. 3.** Maximal aortic root dimension (AOR) plotted against gestational age ( $n = 64$ ,  $y = 0.023x - 0.030$ ,  $r = 0.744$ ,  $p = 0.0001$ ).



**Fig. 4.** Maximal left ventricular internal dimension (LV) plotted against biparietal diameter (BPD) ( $n = 47$ ,  $y = 0.157x - 0.133$ ,  $r = 0.843$ ,  $p = 0.0001$ ).

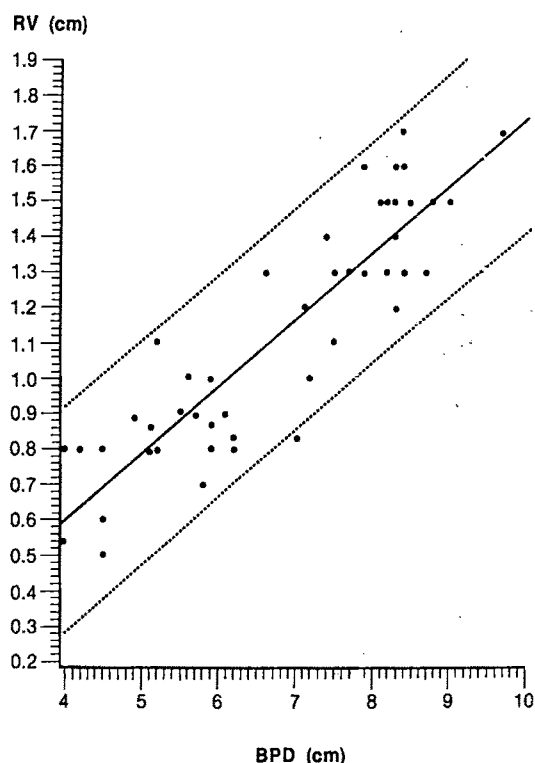


Fig. 5. Maximal right ventricular internal dimension (RV) plotted against biparietal diameter (BPD) ( $n = 46$ ,  $y = 0.189x - 0.158$ ,  $r = 0.895$ ,  $p = 0.0001$ ).

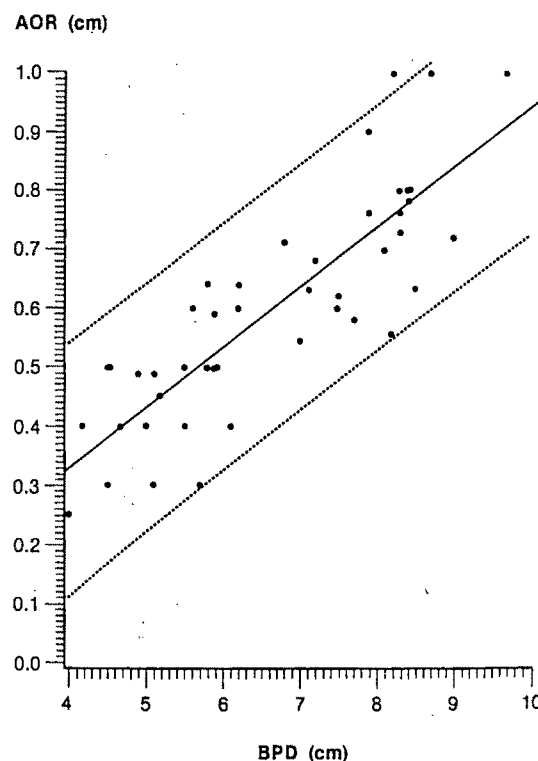


Fig. 6. Maximal aortic dimension (AOR) plotted against biparietal diameter (BPD) ( $n = 46$ ,  $y = 0.102x - 0.081$ ,  $r = 0.847$ ,  $p = 0.0001$ ).

the fetus died during preparations for emergency cesarean delivery. Retrospective analysis of the video-recorded images revealed the left ventricular and left atrial measurements to be 1.7 and 1.6, respectively. The corresponding right ventricular and right atrial measurements were 2.2 and 2.1. Postmortem findings confirmed our diagnosis of four-chamber enlargement. The heart was pale and flabby. There was no apparent cause of this myocardial failure.

**Case 5.** During the active phase of labor, this fetus experienced brief early decelerations, which became more profound in the second stage, with intermittent delayed recovery times. Echocardiography revealed a structurally normal heart with normal dimensions. However, mild to moderate hypokinesis was noted during contractions, with a return to normal wall motion between contractions. The percentage of systolic fractional shortening for the left ventricle was 40% between contractions and fell to 21% during contractions. At birth, the cord was tightly wrapped around the neck. Apgar scores were 1 and 7 at 1 and 5 minutes, respectively. The neonate required intubation and transfer to the neonatal intensive care unit where steady improvement occurred during the next few days.

#### Comment

When we first began measuring cardiac dimensions, our success rates were as follows: left ventricle and right

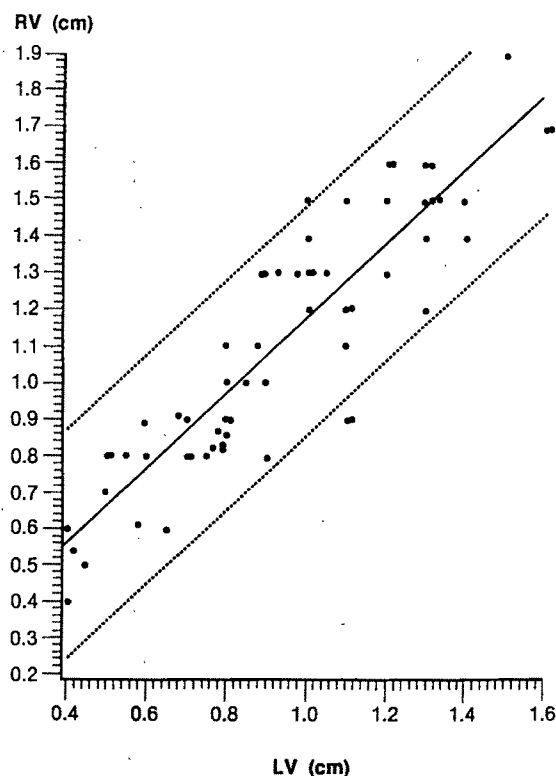


Fig. 7. Maximal right ventricular internal dimension (RV) plotted against maximal left ventricular internal dimension (LV) ( $n = 62$ ,  $y = 1.03x + 0.144$ ,  $r = 0.899$ ,  $p = 0.0001$ ).

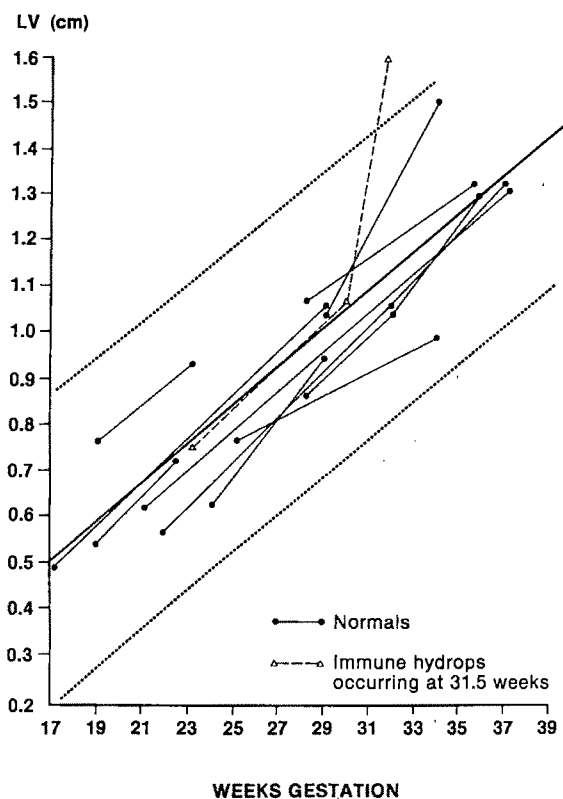


Fig. 8. Maximal left ventricular internal dimensions (LV) in serially monitored patients.

ventricle, 87%; left atrium and right atrium, 47%; and aortic root, 85%. Experience has enabled us to achieve, at this time, more than 90% success for all dimensions. Major limiting factors are obesity, fetal activity, and unusual fetal lie. Fetal activity problems can be overcome by simply waiting for the fetus to rest, while an unusual lie can sometimes be dealt with by repositioning the transducer and/or the patient. Our data are comparable to those obtained by M-mode studies<sup>1,2</sup> in terms of absolute values and regression slopes. The exception is our finding of right heart dominance, whereas the M-mode data report a right ventricular/left ventricular ratio of unity. Several conflicting reports exist concerning the relative sizes of the two ventricles.<sup>6-8</sup> Recent data obtained from mature fetal lambs place the right ventricular and left ventricular components of cardiac output at 60% and 40%, respectively.<sup>9</sup>

There are several advantages to obtaining M-mode studies. These include sharply defined endocardial surfaces and the ability to clarify arrhythmias. As well, since end-diastolic and end-systolic measurements can be accurately measured, one can estimate systolic shortening. Formulas exist for using these measurements to calculate stroke volume.<sup>2, 10</sup> Hence, cardiac output might be noninvasively measurable. However, since the small fetal diameters used in these formulas must be

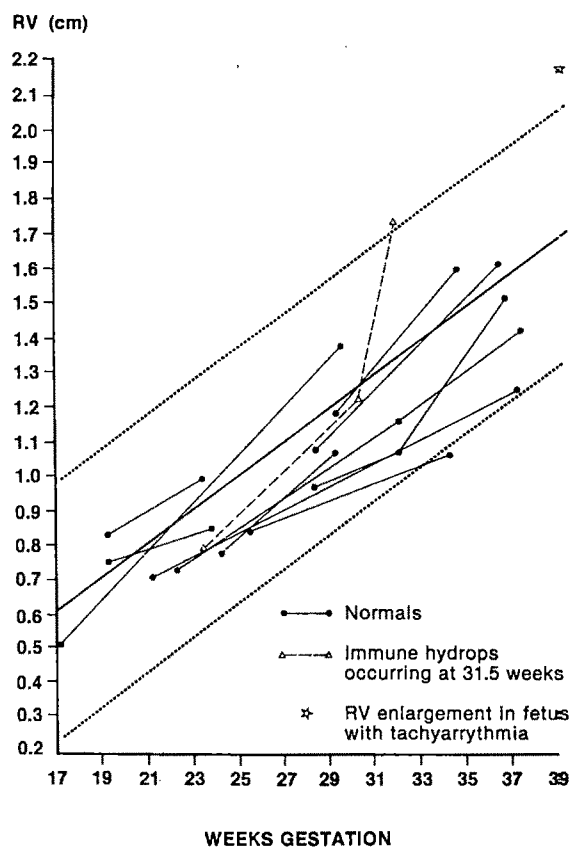


Fig. 9. Maximal right ventricular internal dimensions (RV) in serially monitored patients. Star symbol in right upper corner represents right ventricular dimension in Case 3.

squared or cubed, one might anticipate a wide margin of error in such calculations.

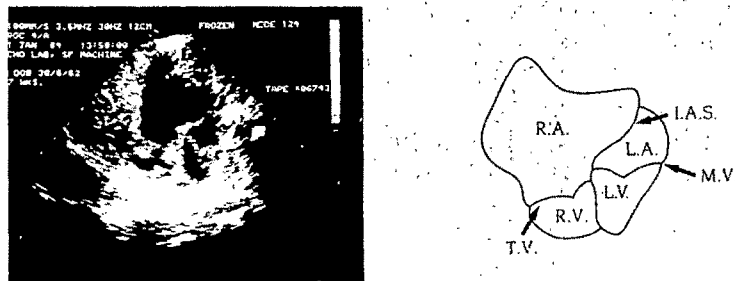
The advantages of obtaining two-dimensional images include the ability to diagnose congenital anomalies, better spatial orientation for more accurate chamber measurements, and direct visualization of wall motion to assess myocardial performance.

Obviously, the two are complementary, and it is now our practice, whenever possible, to obtain both two-dimensional and M-mode information (Figs. 11 and 12).

We feel that the establishment of standard measurements and growth curves invites a number of clinical applications. Abnormal chamber size may be the first clue to congenital defects, such as Ebstein's anomaly or hypoplastic ventricles. Since early medical or surgical treatment (for example, prostaglandin infusion in Case 2) might alter the prognosis, an in utero diagnosis of congenital heart disease should prompt the transfer of the gravid patient to a center where cardiovascular services would be quickly available.

Quantitative echocardiography can also be used to assess the fetus at risk for cardiomegaly and congestive heart failure (for example, immune hydrops, tachyar-

# EBSTEIN'S ANOMALY



**Fig. 10.** Case 2. Fetus with Ebstein's anomaly, showing large right atrium (diameter 6.1 cm) and apically displaced tricuspid valve.

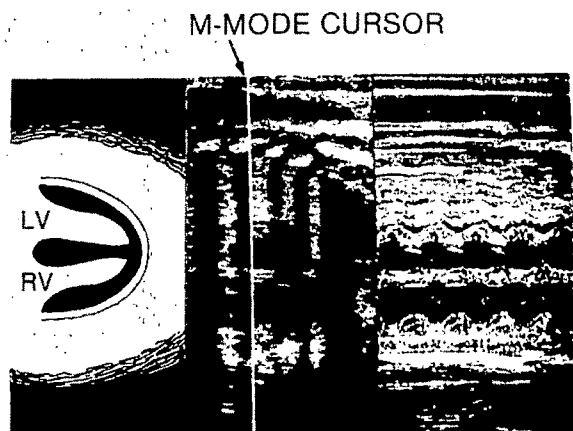


**Fig. 11.** Four-chamber view of fetal heart. Dotted line indicates plane of measurement for maximal ventricular dimensions.

rythmias, and twin-to-twin transfusion) and to monitor the response to therapy (for example, intrauterine transfusions or cardioversion in utero).

Another potential application of our data is illustrated by Cases 4 and 5, which likely represent different degrees of the same problem, namely, fetal asphyxia. The initial fetal response to hypoxia or mild asphyxia consists of selective vasoconstriction, hypertension, and bradycardia. As the asphyxia worsens, cardiac performance diminishes, hypotension follows, and the umbilical circulation becomes compromised.<sup>11</sup> In Case 4, we observed the echocardiographic changes of terminal asphyxia. In Case 5, the fetus manifested a hypoxic-bradycardiac response during contractions but recovered between contractions. We were able to demonstrate echocardiographically a significant decrease in the percentage of fractional systolic shortening of the left ventricle during contractions. It is likely, therefore, that the fetal stroke volume and cardiac output diminished with uterine contractions. This effect might be explained by an increase in afterload on the left ventricle as a result of systemic hypertension induced by hypoxia.<sup>6, 12</sup>

Bradycardia alone is not an accurate gauge of the



**Fig. 12.** M-mode tracing of fetal heart, obtained by placing the cursor at the plane indicated in the adjacent line drawing.

level of hypoxia<sup>12</sup> and, since fetal blood gases and pH are not always measurable, an alternate means of assessing the degree of fetal compromise could be an extended cardiovascular profile.

Recent reports<sup>13-16</sup> describing the Doppler evaluation of uterine, umbilical, and fetal aortic blood flow, combined with our ability to measure cardiac chambers and

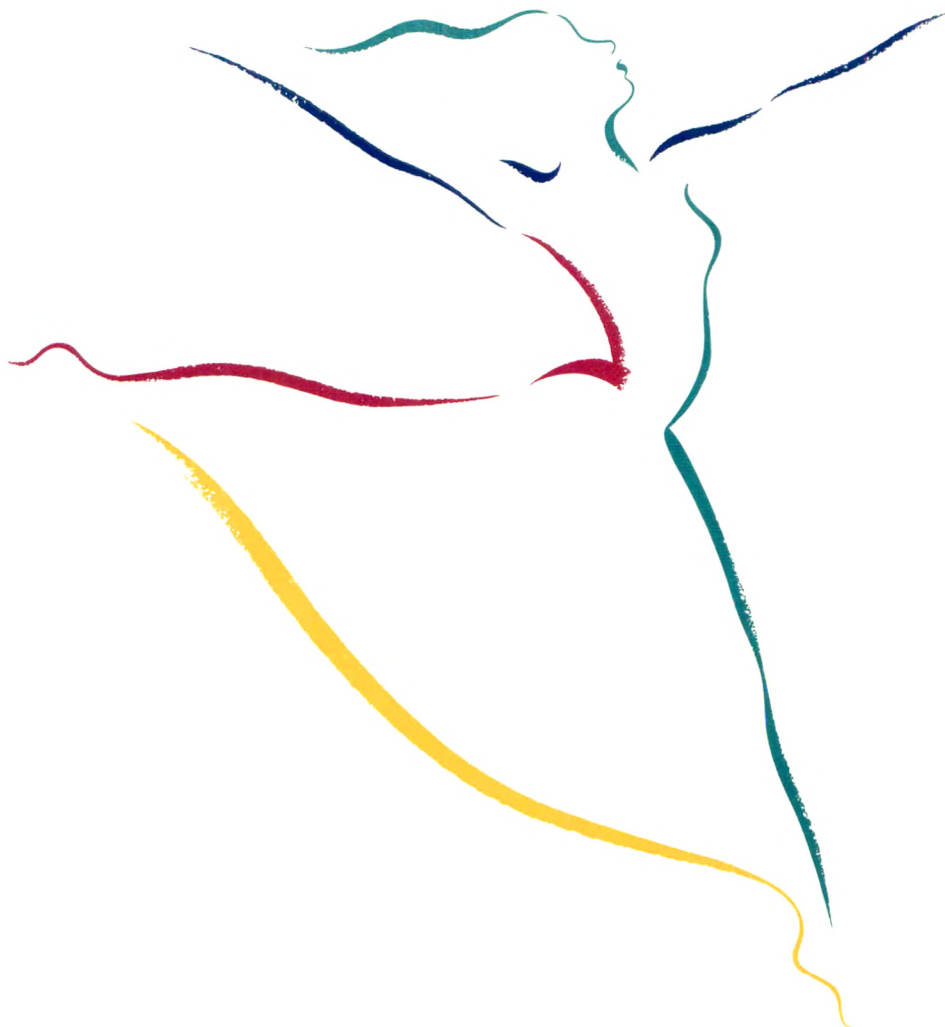


wall motion (systolic shortening), are promising new starts in this direction. With echocardiography, future questions to be addressed are more of a physiologic than a technical nature. For example, to what extent do normal cardiac function and size guarantee fetal health? How soon after the onset of hypoxia might we observe changes, and do these changes measurably correlate with the degree of the insult? Once cardiac enlargement and diminished wall motion are observed, what time frame is available before intervention must occur? What is the myocardial response to oxygen administration and/or pharmacologic preparations?

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New imidazole for vulvovaginal candidiasis



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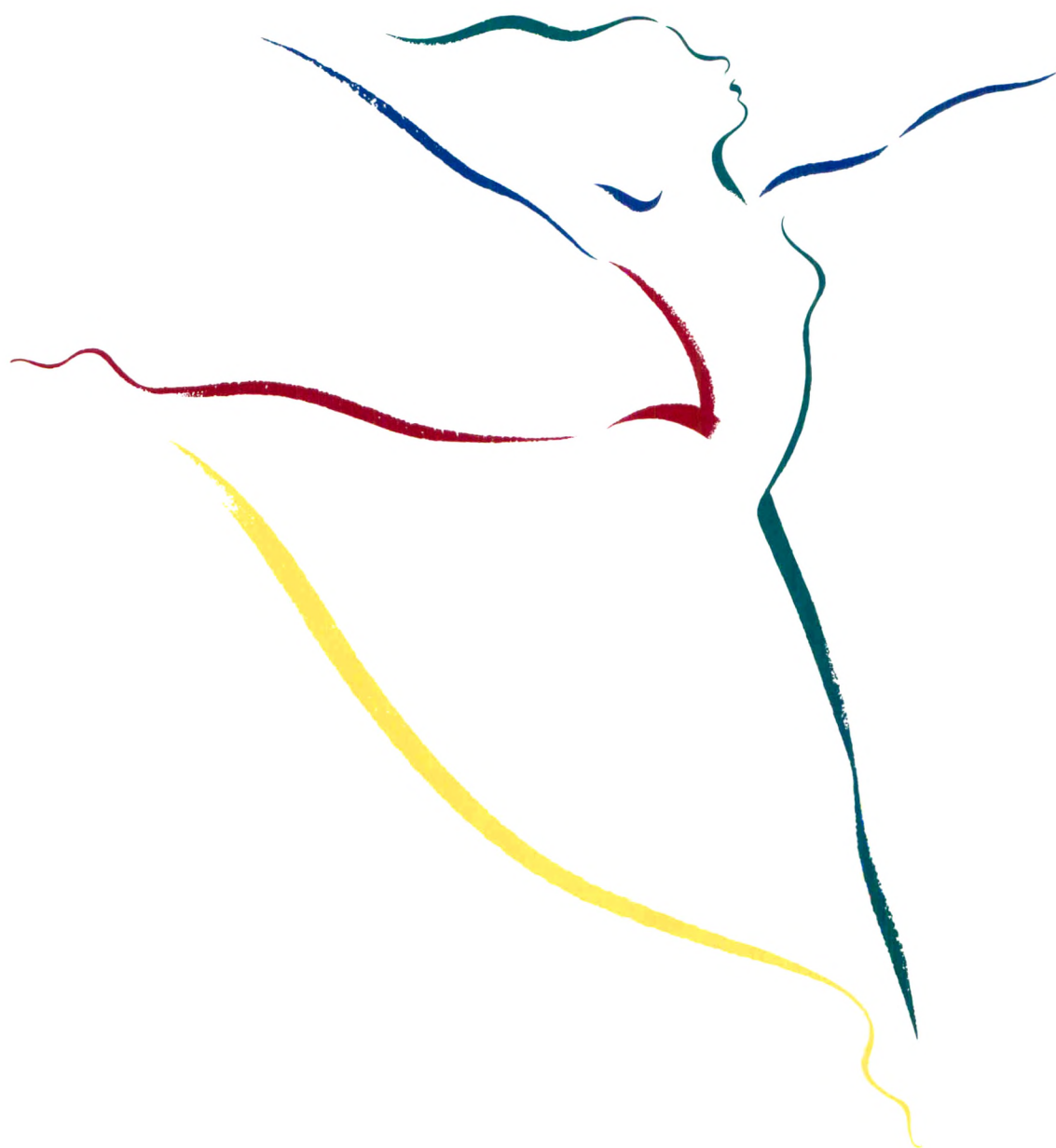
\*In non-pregnant patients, 3-day treatment is recommended. Treatment may be extended an additional 3 days, if necessary.  
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New imidazole for  
vulvovaginal candidiasis

# 3-Day Cream

The shortest course of the preferred form<sup>1</sup>



New  
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**Femstat**<sup>®</sup>  
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**The Only 3-Day Cream**

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  - ≡ Cream—the preferred form for prompt relief of vulvar itching and burning.<sup>1</sup>
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- 

1. According to a 12-month nationwide industry audit (ending October 1985), comprised of approximately 2,000 pharmacies. Vulvar burning occurred in 2.3% and itching in 0.9% of patients on Femstat.

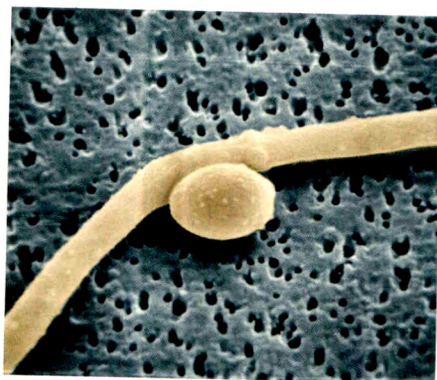
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# New imidazole for vulvovaginal candidiasis

## New Femstat<sup>®</sup> vaginal cream 2.0% (butoconazole nitrate) The Only 3-Day Cream



Scanning electron photomicrographs (5000X) of *Candida albicans* grown for 48 hours in serum (left), and in serum with 200 mcg/ml butoconazole nitrate (right).

### Brief Summary

#### INDICATIONS:

FEMSTAT<sup>®</sup> (butoconazole nitrate) vaginal cream 2.0% is indicated for local treatment of vulvovaginal mycotic infections caused by *Candida* species. Confirm the diagnosis by KOH smears and/or cultures. FEMSTAT can be used with oral contraceptives and antibiotics. It is effective in non-pregnant women and during the second and third trimesters of pregnancy.

#### CONTRAINDICATIONS:

FEMSTAT is contraindicated in patients hypersensitive to any of the ingredients.

#### PRECAUTIONS:

##### General:

If clinical symptoms persist, repeat microbiological tests to rule out other pathogens and confirm the diagnosis. Discontinue drug if sensitization or irritation occurs.

##### Information for the Patient:

Do not discontinue prematurely during menstruation or because of symptomatic relief.

##### Carcinogenesis:

Animal studies have not been done.

##### Mutagenesis:

Mutagenicity studies were negative.

##### Impairment of Fertility:

Animal studies showed no impairment of fertility.

#### Pregnancy Category C:

Adverse effects were noted in animals treated with high oral doses. No studies were done in women during first trimester. Patients in the second or third trimester have shown no adverse effects attributable to the drug.

#### Nursing Mothers:

Use with caution.

#### Pediatric Use:

Safety and efficacy have not been established.

#### ADVERSE REACTIONS:

Vulvar/vaginal burning in 2.3% of patients, vulvar itching in 0.9%, discharge, soreness, swelling, itching of fingers each in 0.2%. Complaints caused 1.6% to discontinue drug.

#### DOSAGE AND ADMINISTRATION:

Non-pregnant Patients: The recommended dose is one applicatorful of cream (approximately 5 grams) intravaginally at bedtime for three days. Treatment can be extended for an additional three days if necessary.

Pregnant Patients (second and third trimesters only): The recommended dose is one applicatorful of cream (approximately 5 grams) intravaginally at bedtime for six days.

#### CAUTION:

Federal law prohibits dispensing without prescription.

December 1985



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# Myometrial desensitization to continuous but not to intermittent $\beta$ -adrenergic agonist infusion in the sheep

Robert F. Casper, M.D., and Stephen J. Lye, Ph.D.

London, Ontario, Canada

Infusion of the long-acting  $\beta$ -adrenergic agonist ritodrine ( $3 \mu\text{g/kg/min}$ ) caused by rapid inhibition of uterine activity in the ovariectomized, nonpregnant sheep. This inhibition could only be maintained for  $6.4 \pm 0.8$  hours, with high-frequency activity returning by  $11.4 \pm 2.6$  hours despite continuous infusion of ritodrine. Intermittent administration of ritodrine did not prolong uterine relaxation, probably as a consequence of its long half-life. Continuous infusion of the short-acting  $\beta$ -agonist isoproterenol ( $0.16 \mu\text{g/kg/min}$ ) initially inhibited uterine contractions but high-frequency activity returned by 50 minutes. In contrast, intermittent infusion of isoproterenol (30 minutes on and 30 minutes off) significantly inhibited the frequency of contractions during each of the infusion periods for the duration of the study (13 hours). Our data demonstrate that either continuous administration of  $\beta$ -agonists or intermittent administration of the long-acting  $\beta$ -agonist ritodrine resulted in myometrial desensitization in the sheep. In contrast, intermittent administration of the short-acting  $\beta$ -adrenergic agonist isoproterenol prevented the onset of myometrial desensitization. (AM J OBSTET GYNECOL 1986;154:301-5.)

**Key words:**  $\beta$ -Adrenergic agonists, uterine contractions, premature labor, nonpregnant sheep

At present the major treatment for preterm labor is the administration of a selective  $\beta_2$ -adrenergic agonist that inhibits myometrial contractility by activation of receptor-mediated adenylyl cyclase leading to increased content of adenosine 3':5'-cyclic phosphate (cyclic-AMP). However, the ability of this therapy to significantly prolong gestation in preterm labor is equivocal.<sup>1,2</sup> Recent reports have shown that continuous exposure of human myometrium to  $\beta$ -adrenergic agonists both in vitro and in vivo results in initial myometrial relaxation followed by desensitization with return of myometrial contractions.<sup>3-5</sup> We have shown that the development of desensitization to a  $\beta$ -adrenergic agonist by human myometrial strips in an organ bath could be prevented by changing from continuous to intermittent exposure to the agonist.<sup>5</sup> In the present study we confirm and extend these observations in vivo with use of a nonpregnant sheep model. Specifically, our aims were to determine whether continuous infusion of a  $\beta$ -adrenergic agonist would result in myometrial desensitization in vivo and to test the hypothesis that desensi-

tization could be prevented by intermittent administration. Since pharmacodynamics become important in vivo, we compared a long-acting (ritodrine) with a short-acting (isoproterenol)  $\beta$ -agonist in both continuous and intermittent modes of administration.

## Material and methods

Eight nonpregnant sheep of mixed breed were bilaterally ovariectomized under general anesthesia. Intrauterine recording balloons made of latex rubber were implanted to record mechanical changes in uterine activity, measured as intrauterine pressure cycles.<sup>6</sup> Pairs of stainless steel electrodes (Cooner Corporation, California) were sutured into the myometrium of each uterine horn as previously described<sup>7</sup> to record electromyographic activity. Intrauterine pressure was measured by connecting the balloon catheters to pressure transducers (Statham Model p 23), and the electromyographic signal was processed through a wide-band a/c preamplifier (Grass Model 7P511J) with use of a one-half amplitude low-frequency filter of 0.3 Hz and a one-half amplitude high-frequency filter of 10 kHz. The external jugular vein was catheterized with use of polyethylene tubing (PE100, Intramedic, Clay Adams, Parsippany, New Jersey) for intravenous infusions. To protect the lines from being pulled out, intrauterine balloon catheters, myometrial electrodes, and the jugular vein catheters were run subcutaneously to emerge from the skin of the flank in each animal. The ewes were housed in metabolic cages and received food and

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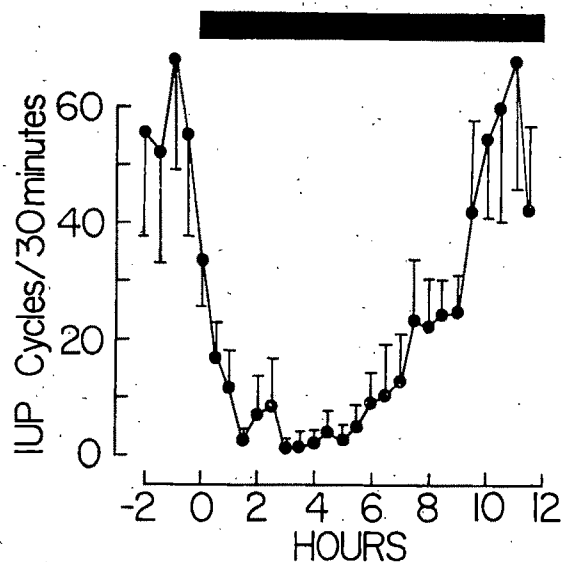


Fig. 1. Continuous infusion of ritodrine in six ovariectomized estrogen-treated nonpregnant sheep. Mean ( $\pm$ SEM) intrauterine pressure (IUP) cycles per 30 minutes are plotted for 2 hours before and 12 hours during the ritodrine infusion.

water ad libitum. Lighting in the animal rooms was on from 6 AM until 8 PM, and temperature was maintained at 22°C.

**Experimental protocol.** 17 $\beta$ -Estradiol was administered to the animals subcutaneously in 0.25 ml of corn oil to induce spontaneous uterine activity as previously described.<sup>8</sup> Continuous recording of intrauterine pressure and electromyographic activity was begun 3 days after the surgical procedure. To investigate the effects of a long-acting (ritodrine) and a short-acting (isoproterenol)  $\beta$ -adrenergic agonist on intrauterine pressure cycles and electromyographic activity, the following two experiments were performed at least 7 days after the operation.

**Ritodrine infusion experiment.** The clinically used  $\beta$ -agonist ritodrine (Bristol Laboratories, Ottawa, Ontario) was infused continuously at a rate of 3  $\mu$ g/kg/min for up to 18 hours in six experiments, and intrauterine pressure cycles and electromyographic activity were recorded continuously. This dosage represents the average dose used clinically to stop preterm labor.<sup>9</sup> We also used several intermittent schedules of administration of ritodrine. These included hourly injection (1.5 mg in 30 seconds), 30 minutes on and 30 minutes off, 60 minutes on and 60 minutes off, and 360 minutes on and 60 minutes off.

**Isoproterenol infusion experiment.** The short-acting,  $\beta$ -adrenergic agonist isoproterenol (Sterling-Winthrop Laboratories, Aurora, Ontario) was infused continuously (0.16  $\mu$ g/kg/min) for up to 8 hours, and intrauterine pressure cycles and electromyographic activity were recorded. To examine the effects of intermittent administration, isoproterenol was infused at the same

rate for only 30 minutes of each hour; that is, the infusion was on for 30 minutes and off for 30 minutes alternately for up to 13 hours. Again, intrauterine pressure cycles and electromyographic activity were recorded continuously.

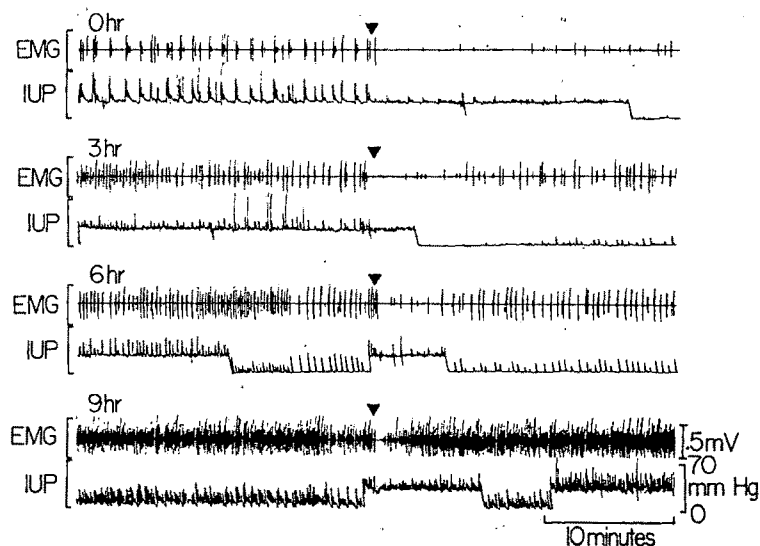
**Analysis of intrauterine pressure records.** Intrauterine pressure records were analyzed as described by Lye and Porter.<sup>6</sup> An intrauterine pressure cycle was defined as an increase and decrease of intrauterine pressure of at least 10 mm of mercury. Intrauterine pressure records were divided into 15-minute periods, and frequency and maximum amplitude of intrauterine pressure cycles were recorded for each period. Statistical analysis was by one-way analysis of variance and Duncan's new multiple range test.

## Results

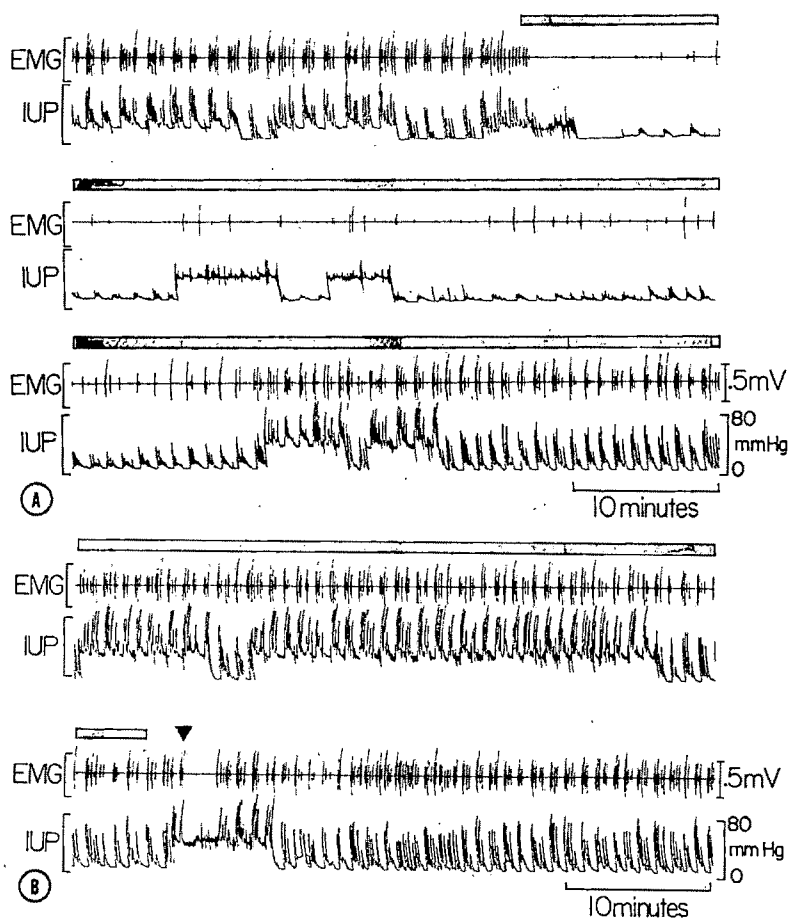
**Ritodrine experiments.** Infusion of ritodrine continuously in 6 animals resulted in a rapid inhibition of the frequency of intrauterine pressure cycles ( $p < 0.001$ ; Fig. 1). The amplitude of intrauterine pressure cycles was also significantly inhibited. Low-frequency uterine activity ( $<20$  intrauterine pressure cycles per 30 minutes) was maintained for  $6.4 \pm 0.8$  hours. Despite continuous infusion of ritodrine, uterine contractions returned and reached a high level of activity ( $>50$  intrauterine pressure cycles per 30 minutes) and high amplitude ( $>40$  mm Hg) by 10 hours.

Infusion of ritodrine by various intermittent schedules failed to significantly prolong the period of inhibition. Bolus injection of ritodrine (1.5 mg/30 sec each hour) in an attempt to establish intermittent blood levels of the agonist also failed to prevent desensitization, as shown in Fig. 2. In this experiment the period of inhibition of electromyographic and intrauterine pressure activity after each bolus of ritodrine became progressively shorter, so that by 9 hours the drug bolus had little effect on uterine activity.

**Isoproterenol experiments.** Infusion of the short-acting  $\beta$ -adrenergic agonist isoproterenol also produced profound initial inhibition of uterine electrical and mechanical activity (Fig. 3, A). However, with continuous infusion, high-frequency electromyographic and intrauterine pressure activity returned by 50 minutes. Desensitization was confirmed during this time by administration of a large bolus dose of isoproterenol (1.5 mg), which failed to inhibit uterine activity for more than 2 minutes (Fig. 3, B). Composite data for six experiments is shown in Fig. 4. Both frequency of intrauterine pressure cycles (from  $37.5 \pm 5.5$  to  $9.2 \pm 3$  cycles per 30 minutes) and amplitude (from  $47.5 \pm 9.5$  to  $22.2 \pm 4.9$  mm Hg) were inhibited significantly 15 minutes after the start of the infusion ( $p < 0.05$ ). Frequency of uterine activity remained low ( $<20$  intrauterine pressure cycles per 30 minutes) until



**Fig. 2.** Electromyographic (EMG) and intrauterine pressure (IUP) recordings in a single sheep treated with hourly bolus injection of ritodrine (1.5 mg/30 sec) indicated by the arrowhead. Changes in intrauterine pressure baseline indicate positional changes of the sheep from standing to lying or vice versa.



**Fig. 3.** A, Electromyographic (EMG) activity and intrauterine pressure (IUP) in a representative experiment recorded for 30 minutes before and during infusion of isoproterenol indicated by the shaded bar. B, Continuation of the recording of electromyographic and intrauterine pressure activity begun in A. Continuous isoproterenol infusion (shaded bar) was terminated, and the sheep was given a large bolus injection of isoproterenol (1.5 mg) intravenously (arrowhead).



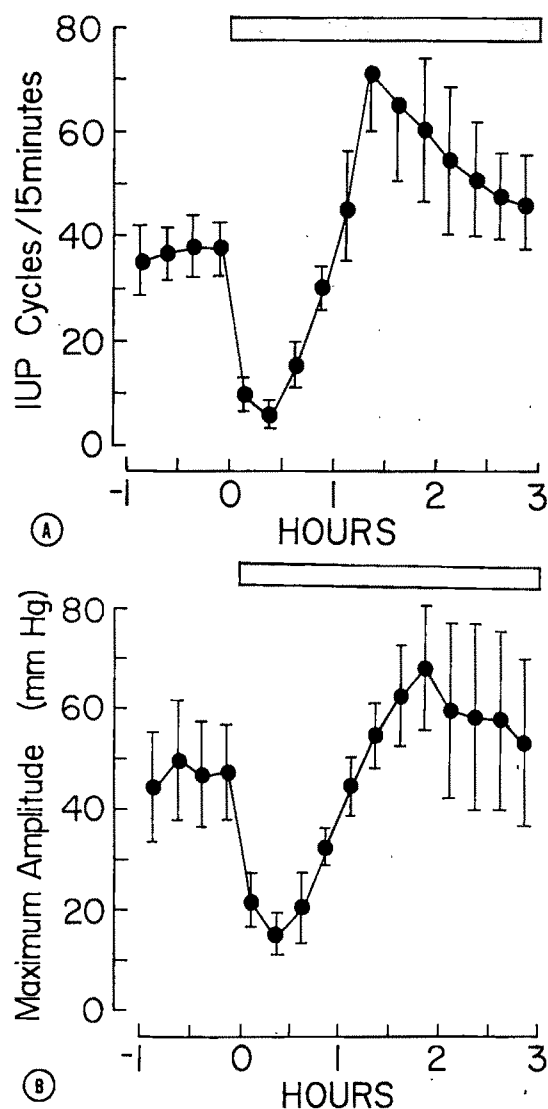


Fig. 4. Composite data for mean ( $\pm$ SEM) frequency (cycles per 15 minutes) (A) and amplitude (mm Hg) (B) of intrauterine pressure (IUP) cycles in 6 isoproterenol infusion experiments. Intrauterine pressure cycles were analyzed for 1 hour before and 3 hours during isoproterenol infusion (shaded bar). Acute inhibition of both frequency and amplitude of intrauterine pressure cycles was seen ( $p < 0.05$ ) at 15, 30, and 45 minutes after the start of infusion, but baseline intrauterine pressure cycle frequency and amplitude were reached by 50 minutes despite continuing isoproterenol infusion.

60 minutes when high-frequency, high-amplitude contractions returned despite continuing infusion of isoproterenol. Once desensitization occurred, the frequency and amplitude of contractions tended to be higher than baseline levels although this increase did not reach significance.

Intermittent infusion of isoproterenol (30 minutes on and 30 minutes off) resulted in a rapid inhibition of both electrical and mechanical activity. In the five animals studied (Fig. 5), the frequency of intrauterine

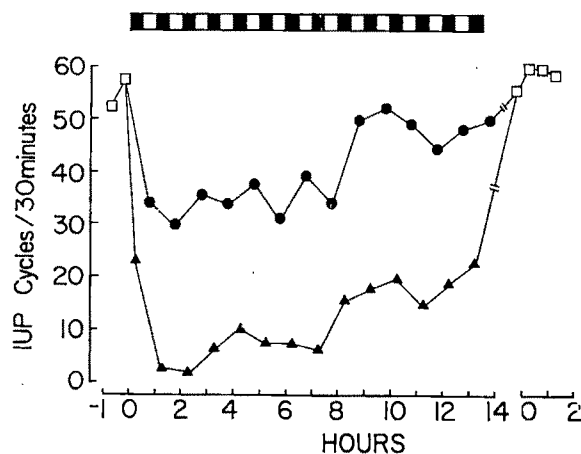


Fig. 5. Mean intrauterine pressure (IUP) cycles in five sheep treated with intermittent isoproterenol infusion for 13 hours. Isoproterenol was infused for 30 minutes (black bars) and then discontinued for 30 minutes (white bars) alternatively. Mean intrauterine pressure cycle frequency during the 30 minutes that isoproterenol infusion was turned off is shown by solid circles. Intrauterine pressure cycle frequency during each 30-minute infusion period (solid arrowhead) was significantly less ( $p < 0.05$ ) than the baseline frequency (open square). Standard error of the mean for each point is not shown.

pressure cycles during each 30-minute infusion period remained significantly ( $p < 0.05$ ) below the frequency before administration of isoproterenol throughout the 13 hours of the experiment. During the 30-minute period when no isoproterenol was infused, intrauterine pressure cycle frequency increased although almost all of this increase occurred during the last 15 minutes of each off period and carried over into the beginning of the next isoproterenol infusion period. The frequency and amplitude of intrauterine pressure cycles rapidly increased following termination of the pulsatile infusion protocol.

### Comment

In this study we demonstrated that continuous infusion of isoproterenol or ritodrine can acutely inhibit uterine activity in the sheep. However, with continuing infusion of both  $\beta$ -agonists, desensitization with return of uterine contractions occurred. The specific mechanism of  $\beta$ -adrenergic agonist-induced desensitization is unclear. There is evidence that catecholamine-induced loss of responsiveness may involve both post-receptor cyclic-AMP-mediated mechanisms (heterologous desensitization) as well as hormone-receptor specific phenomena such as receptor internalization which is unrelated to elevation of cyclic-AMP (homologous desensitization) (see Reference 10 for review).

In a study by Berg et al.<sup>4</sup> myometrial biopsies from women treated with terbutaline for at least 2 weeks and in whom labor could not be arrested were shown to have lower basal cyclic-AMP content compared with

untreated control subjects. In addition, the cyclic-AMP content of these myometrial samples did not exhibit the expected increase when stimulated by  $\beta$ -adrenergic agonists in vitro. More recently the same authors<sup>11</sup> have demonstrated that the concentration of  $\beta$ -adrenergic receptors in the uterine fundus of women treated with terbutaline was 50% of that found in untreated women. These data suggest that  $\beta$ -adrenergic desensitization in the human myometrium may, at least in part, be due to a receptor-specific mechanism.

Our previous report<sup>5</sup> of the effect of isoproterenol on human myometrial strips in vitro suggests that after desensitization has occurred, rapid recovery of responsiveness can be achieved by removing the  $\beta$ -agonist from the medium. In the present study we attempted to maintain the sensitivity of the myometrium to the relaxing effects of  $\beta$ -agonists in vivo by intermittent infusion of these agents. It quickly became apparent, however, that the long half-life of ritodrine (approximately 4 hours<sup>12</sup>) made it impossible to establish interrupted exposure of the myometrium to this agent with "infusion off" times as long as 60 minutes. Even bolus injection of ritodrine each hour resulted in complete myometrial desensitization with a time course about the same as continuous infusion. It is possible that interrupting the ritodrine infusion for a period of time longer than the half-life of the drug may reestablish myometrial sensitivity and a return of the inhibitory effect of the infusion. In contrast, the short-acting  $\beta$ -adrenergic agent isoproterenol when infused for 30 minutes of each hour was able to maintain sensitivity of the myometrium for the entire length of the experiment (up to 13 hours). However, the return of contractions between the infusion periods suggests that the schedule of 30 minutes on and 30 minutes off is not ideal. It is likely that shorter off periods with or without shortening of the on period may be necessary to more effectively inhibit uterine activity. These findings thus confirm our hypothesis that intermittent administration of a  $\beta$ -agonist in vivo can prevent myometrial desensitization in the nonpregnant sheep. Additional experiments are required to determine the optimal schedule of  $\beta$ -agonist infusion to inhibit myometrial contractility without producing desensitization. Al-

though it remains to be seen whether these data can be extrapolated to pregnant animals, we have demonstrated that myometrial desensitization occurred when ritodrine was infused continuously to pregnant ewes in whom preterm labor was induced with adrenocorticotrophic hormone (Lye S, Mitchell B, Casper R, unpublished observations). We are now investigating the ability of intermittent  $\beta$ -agonist infusion to inhibit preterm labor induced in this sheep model.

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# An analysis of the effects of increasing doses of ionizing radiation to the exteriorized rat ovary on follicular development, atresia, and serum gonadotropin levels

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There is increasing interest in the effects of environmental and therapeutic agents on the reproductive system, in particular, the ovary. To study the effects of controlled doses of ionizing radiation to the ovary, Sprague-Dawley rats had their ovaries exteriorized and subjected to increasing doses of radiation. There was a significant increase in ovarian follicular atresia, a significant increase in serum follicle-stimulating hormone levels, but no change in serum luteinizing hormone levels. This experimental protocol may facilitate the testing of putative radioprotectants. (AM J OBSTET GYNECOL 1986;154:306-9.)

**Key words:** Radiation, ovary, atresia, menopause

There is increasing interest in the effects that various environmental and therapeutic agents have on the reproductive capacity of mammals.<sup>1,2</sup> One major agent that has profound effects on ovarian follicular development, fertilization, and early development is ionizing radiation.<sup>3</sup> Despite many studies describing the effects of radiation in a variety of experimental systems, the exact mechanism of cell injury and death has not been completely determined, particularly with respect to endocrine tissues.<sup>3,5</sup>

It was the specific intention of these investigations to determine the *in vivo* endocrine responses of the rat ovary to varying doses of ionizing radiation. In the present study the ovarian response was measured in terms of follicular, antral, and preantral atresia. These morphologic changes were compared to serum gonadotropin levels.

## Material and methods

Sprague-Dawley rats were purchased from the Charles River Company, given food and water *ad libitum* and maintained on a 14-hour light/10-hour dark cycle. On day 30 the rats were anesthetized with 0.1 mg/kg of pentobarbital, the abdomen opened with a midline incision, the ovaries identified, and the ovarian

arteries ligated with a Weck clip. The vascular supply to the ovaries from the uterus was maintained. The rats were placed in a lead box, 1 cm thick, with an aperture to permit the ovaries to be secured in the radiation beam. A General Electric Maxitron 250 was used at 250 kV with a current of 30 mA and with 0.1 mm aluminum and 0.5 mm copper filters. The ovaries were 17 cm from the focal point of the tube, and the final dose rate was approximately 1000 rad/min. Control animals underwent ovarian artery ligation and exteriorization with sham irradiation. The ovaries were returned to the abdominal cavity, which was closed with silk suture. The numbers of animals per group were control, 4; 2000 rad, 5; 3000 rad, 5; 4000 rad, 4; 5000 rad, 5; 6000 rad, 5.

On day 44 the animals were killed by cervical dislocation and decapitation, and the wet weight of the ovaries and uteri was recorded. The ovaries were fixed in Davidson's medium, dehydrated in increasing concentrations of alcohol, embedded in paraffin, sectioned at 7  $\mu$ m through the entire ovary, and stained with hematoxylin and eosin. Every fifth section was assessed for the presence or absence of atresia in all antral and preantral follicles containing an oocyte. Assessment of atresia was done with use of the method of Byskov<sup>6</sup> and quantified with the Bioquant Digitizing System on a Saccata SG 1000 High Resolution Video Display on a Teo Tiger 2000 computer.

The trunk blood was collected for the measurement of serum luteinizing hormone and follicle-stimulating hormone by a double-antibody radioimmunoassay technique validated in our laboratory by Kitchen et al.<sup>7</sup> Serum estradiol and progesterone were measured by specific radioimmunoassay.<sup>8</sup>

Statistical significance was accepted at  $p < 0.05$ . For

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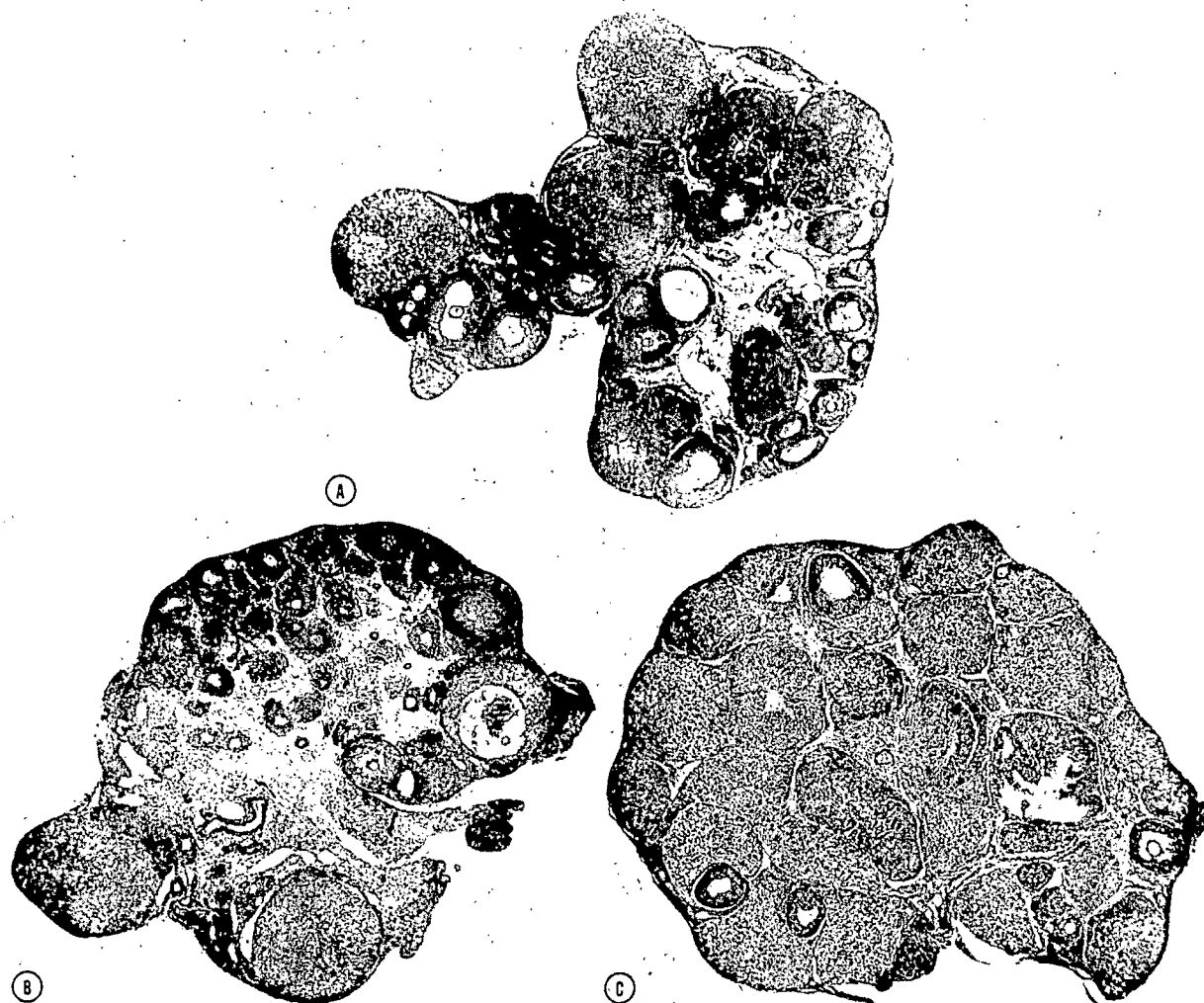


Fig. 1. Representative section of ovaries that have been exteriorized and treated with 0 (A), 3000 (B) and 5000 (C) rads on day 35 with decapitation of animals on day 44. Increasing doses of radiation cause a loss in ovarian follicles and an increase in luteinized tissues.

nonparametric data (date of vaginal opening), the Kruskal Wallis and Mann Whitney tests were used; for parametric data, the analysis of variance (one-way) and Student's *t* test were used.

### Results

Four of 32 animals died during the procedure. None developed radiation toxicity. Once vaginal opening occurred between days 34 to 41, estrus smears were obtained from all animals before death on day 44. There was no difference in the mean day of vaginal opening although there was a tendency for opening to be delayed with high doses of radiation ( $p = 0.15$ ). The organ weights are presented in Table I. Increasing doses of radiation produced a significant decrease in ovarian weight represented either as wet weight or wet weight per 100 gm of body weight ( $p < 0.001$ ). Despite the marked reduction in ovarian weight, there was no change in uterine weight in response to radiation, rep-

resented as wet weight or wet weight per 100 gm of body weight ( $p > 0.05$ ) (Table I).

Histologically, there were dramatic changes in the overall structure of the ovary in response to radiation. As shown in Fig. 1, there is a marked loss of ovarian follicles associated with marked increases in areas of luteinized tissues. Quantitatively there was a reduction in the mean number of healthy antral follicles ( $p < 0.05$ ) with no difference in healthy preantral follicles ( $p > 0.05$ ) (Table II). The number of atretic preantral follicles rose abruptly with radiation ( $p < 0.05$ ), yet the number of atretic antral follicles rose to a lesser degree ( $p = 0.11$ ).

The actual rates of atresia per treatment group rose from 62% to 94.4% in antral follicles ( $p = 0.019$ ) and from 21.6% to 77.7% in preantral follicles in a dose-related fashion ( $p = 0.009$ ) (Fig. 2). The increase in atresia became significant at a dose of 2000 rad ( $p < 0.02$ ) (Fig. 2).



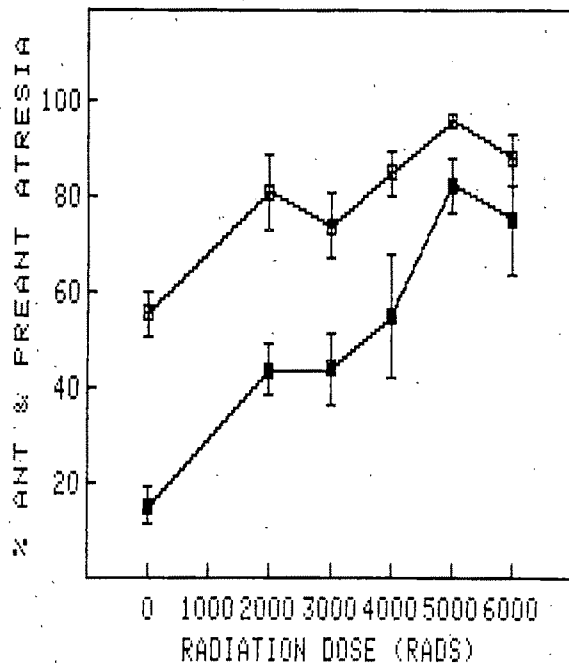


Fig. 2. Increasing doses of ionizing radiation produce significant increases in antral (open box) and preantral (closed box) follicles.

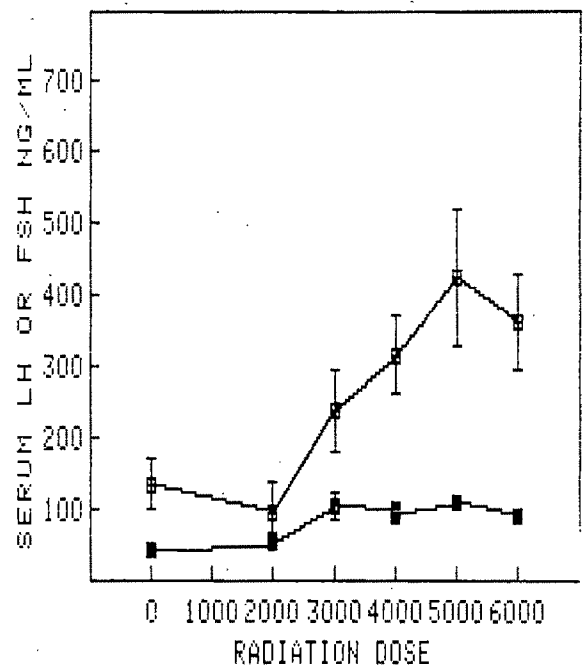


Fig. 3. Increasing doses of radiation produce a rise in serum follicle-stimulating hormone (open box) but no change in serum luteinizing hormone (closed box).

Table I. The effect of radiation dose on ovarian and uterine weights

Dose (rad)	Ovaries (paired)		Uterine weight	
	Wet weight (mg)	Weight (mg)/100 gm of body weight	Wet weight (mg)	Weight (mg)/100 gm of body weight
Control	51.2 ± 2.7*	35.9 ± 1.8†	349.1 ± 81.8‡	174.6 ± 68.7‡
2000	47.5 ± 3.6	31.4 ± 2.5	308.5 ± 17.2	205.1 ± 15.9
3000	38.3 ± 3.3	26.5 ± 2.3	330.1 ± 50.5	229.6 ± 36.6
4000	34.7 ± 5.9	25.3 ± 5.0	316.7 ± 63.1	229.3 ± 49.6
5000	25.7 ± 4.0	17.1 ± 2.7	287.7 ± 21.3	189.8 ± 17.4
6000	29.5 ± 3.0	20.4 ± 2.1	311.7 ± 29.1	215.6 ± 22.1

\* $p < 0.001$ .

† $p < 0.005$ .

‡ $p > 0.05$ .

There was a dose-related increase in serum follicle-stimulating hormone levels in response to increasing doses of radiation ( $p < 0.001$ ). The effects of radiation differ from control animals significantly at the 4000 rad dose but not at the 3000 or 2000 rad dose. There was no significant change in serum luteinizing hormone values in response to radiation ( $p > 0.05$ ) (Fig. 3). There was no difference between groups with respect to serum estradiol or progesterone levels ( $p > 0.05$ ) (Table III).

#### Comment

The experimental design of irradiation of the exteriorized ovary produced results that are consistent with previous reports in which the irradiated animals had a reduction in ovarian weight but maintenance of uterine weight and retention of vaginal cyclicity.<sup>9</sup> The source

of estrogen postirradiation for the maintenance of uterine weight has been attributed to the interstitial cells of the ovary.<sup>3, 10, 11</sup>

With increasing doses of radiation there was an abrupt increase in luteinized tissue, perhaps as a consequence of the luteinization of unruptured follicles at high doses. Although radiation is a reported cause of ovarian follicular atresia, interesting observations can be made with respect to the relative sensitivity of the rat to increasing doses of radiation. The administration of 2000 rad to the exteriorized ovary produced a significant increase in rates of atresia in antral and preantral follicles, a significant loss in the numbers of healthy antral follicles, and a significant increase in the number of atretic preantral follicles, despite the fact that serum follicle-stimulating hormone values remained normal. Only at higher radiation doses did the serum follicle-

**Table II.** Total number of antral and preantral follicles demonstrating oocyte in every fifth section

Dose (rad)	Antral		Preantral	
	Healthy	Atretic	Healthy	Atretic
Control	62.5 ± 6.5*	102.0 ± 7.4*	59.0 ± 5.1*	16.2 ± 1.4
2000	30.7 ± 9.9	156.0 ± 29.1	50.0 ± 6.1	42.0 ± 9.8
3000	47.4 ± 15.7	122.4 ± 24.3	41.4 ± 12.7	29.6 ± 6.8
4000	34.4 ± 17.1	103.8 ± 26.4	42.8 ± 15.5	28.6 ± 4.6
5000	2.5 ± 1.0	61.5 ± 22.5	9.5 ± 3.6	39.0 ± 8.6
6000	5.0 ± 1.6	84.6 ± 56.2	7.0 ± 2.4	24.4 ± 8.0

\*p < 0.05.

stimulating hormone value increase significantly. There is evidence that the serum levels of follicle-stimulating hormone are regulated by a soluble substance, inhibin, from components of the ovarian follicle.<sup>12,13</sup> These findings suggest the possibility that a critical number of follicles must be destroyed before inhibin production is reduced sufficiently to facilitate a rise in serum follicle-stimulating hormone levels.

The corollary to this observation, the significant inhibition of serum follicle-stimulating hormone levels, has been demonstrated by Hodger<sup>14</sup> in rhesus monkeys exposed to human menopausal gonadotropin: It has been hypothesized that superovulation acts to block the estradiol-mediated luteinizing hormone/follicle-stimulating hormone surge via large amounts of inhibin produced in the large numbers of ovarian follicles.<sup>14</sup> The absence of a significant rise in serum luteinizing hormone in rats receiving even 6000 rad to the exteriorized ovaries reflects the continued secretion of estradiol- and progesterone-inhibiting luteinizing hormone release, possibly from the theca cells.<sup>8</sup> The cellular origin of the steroid production at present is speculative however.

With the increased survival of women from a variety of malignant conditions requiring radiotherapy, there is increasing interest in agents that act as radioprotectants in addition to agents that are reported to protect the ovaries from various forms of chemotherapy.<sup>15,16</sup> This model of radiation to the exteriorized ovary, which produces dose-related increases in serum follicle-stimulating hormone and antral and preantral atresia and a dose-related decrease in ovarian weight, may prove to be a good model to test putative radioprotectants. It would appear that a dose of 3000 rad to the exteriorized ovary provides a midrange dose to test such protective agents. Extrapolation of this model to the human may be limited, however, by the nature of steroid production in the presence of widespread atresia.

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**Table III.** The effects of ionizing radiation on serum estradiol and progesterone

Dose (rad)	Estradiol (pg/ml)	Progesterone (mg/ml)
Control	49.67 ± 10.13	11.02 ± 1.10
2000	65.90 ± 32.51	11.58 ± 1.80
3000	56.09 ± 13.96	16.04 ± 2.04
4000	35.92 ± 3.61	11.75 ± 2.90
5000	40.10 ± 3.94	10.66 ± 1.56
6000	45.58 ± 8.53	12.49 ± 2.39

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# Effect of hexoprenaline on uteroplacental blood flow in the pregnant rat

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The effect of  $\beta$ -adrenergic agonists on uteroplacental blood flow is controversial. Human studies, with the use of indirect methods to assess uteroplacental blood flow, show conflicting results. Animal studies in the near-term pregnant sheep model have the disadvantages that the sheep has a syndesmochorial placenta and that the uteroplacental vessels are thought to be maximally dilated near term. The effect of hexoprenaline, a new  $\beta_2$ -sympathomimetic drug, was assessed in the awake pregnant rat on day 14 of gestation by means of the radionuclide-labeled microsphere method. Hexoprenaline increased placental blood flow by 198% and distribution of cardiac output to the placentas by 229%. Renal blood flow was reduced by 24%. Saline solution administration produced no significant effects. (AM J OBSTET GYNECOL 1986;154:310-4.)

**Key words:** Uteroplacental blood flow, hexoprenaline,  $\beta$ -adrenergic agonists

The effect of  $\beta$ -adrenergic stimulants on uteroplacental blood flow in pregnancy remains controversial. Animal studies, mainly with the use of the near-term pregnant sheep model, have generally failed to show an increase in uteroplacental blood flow after administration of these drugs. However, this model has two main disadvantages: (1) The uteroplacental vessels of the near-term pregnant sheep are thought to be maximally dilated and therefore further increases would not be expected; (2) the sheep has a syndesmochorial placenta, unlike the rhesus monkey, rabbit, rat, and man, which have hemochorial placentas.

In the human, various techniques to measure uteroplacental blood flow have been used. However, these methods only indirectly reflect uteroplacental blood flow and have produced conflicting results. Decreased placental perfusion may result in intrauterine growth

retardation and increased fetal morbidity and mortality.

The various  $\beta$ -adrenergic agonists differ as to their receptor selectivity, placental passage, and cardiovascular effects. Based on equivalent tocolytic dosages, hexoprenaline was found to have less effect on maternal heart rate than either fenoterol, ritodrine, or salbutamol.<sup>2</sup> Use of carbon 14-labeled hexoprenaline failed to show any significant placental passage in pregnant rabbits.<sup>3</sup> To our knowledge the effect of hexoprenaline on uteroplacental blood flow has not been previously assessed. The fully conscious rat model at day 14 of gestation (term being 21 days) was chosen for this study as it has a hemochorial placenta and uteroplacental blood flow increases markedly during the last week of pregnancy in this animal.

## Methods

Timed pregnant Sprague-Dawley rats were obtained from Zivic-Miller Laboratories, Inc. (Allison Park, Pennsylvania) and were housed individually in stainless steel wire-bottom cages. Experiments were conducted on 13 rats at day 14 of gestation.

An 8 cm segment of polyethylene catheter (0.28 mm inside diameter by 0.61 mm outside diameter) was in-

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**Table I.** Maternal and fetal weights

Variable	Hexoprenaline	Placebo	p Value
Maternal weight (gm)	334.6 $\pm$ 32.1	306.8 $\pm$ 39.5	0.19
Fetal weight (gm)	2.34 $\pm$ .36	1.94 $\pm$ .44	0.28
Litter size	13.6 $\pm$ 1.9	12.2 $\pm$ 2.6	0.28

Values are mean  $\pm$  SD.

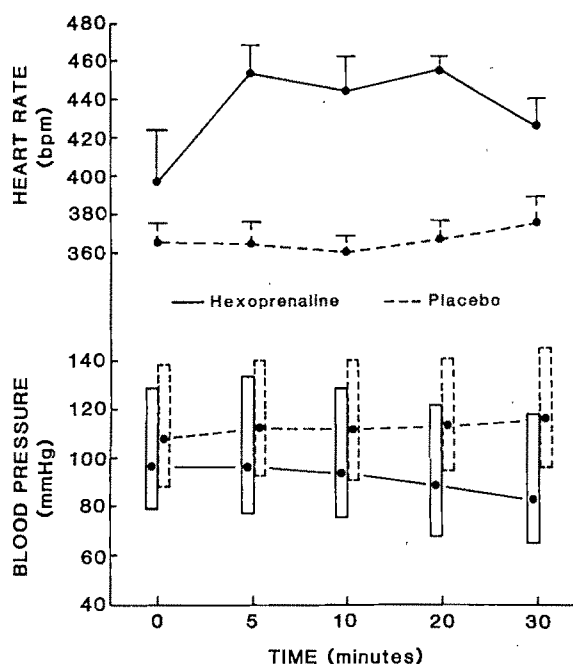


Fig. 1. Effects of hexoprenaline and saline solution on maternal heart rate and blood pressure. Heart rate is shown as mean  $\pm$  SEM. Blood pressure is shown as systolic, mean, and diastolic pressures.

serted into the end of a 30 cm segment of another polyethylene catheter (0.58 mm inside diameter by 0.97 mm outside diameter) and fused. With the animal under ether anesthesia, the small end of the catheter was inserted into the right carotid artery and advanced into the left ventricle. Placement of the catheter tip in the left ventricle was confirmed by the left ventricular pressure pulse tracing, and exact position was verified at autopsy. A similarly prepared catheter was inserted into the right external jugular vein and advanced 2 cm. A third catheter (0.58 mm inside diameter by 0.97 mm outside diameter) was inserted into the left femoral artery. The catheters were filled with heparinized saline solution (20 IU/ml) to prevent clotting and were then tunneled subcutaneously to a small hole in the skin at the back of the neck. From the exit point, the catheters were passed through a small metal spring to ensure that the animal would not chew on them. The animal was then placed in a holding cage and allowed a 3-hour recovery period. The femoral catheter was connected to a Statham P23Gb pressure transducer and arterial pressure and heart rate were recorded on a Gould 2200S recorder. The transducer was then disconnected and the catheter was attached to a constant withdrawal syringe pump (Harvard Apparatus, South Natick, Massachusetts), which was used to draw the arterial reference sample.

A suspension of approximately 100,000 micro-

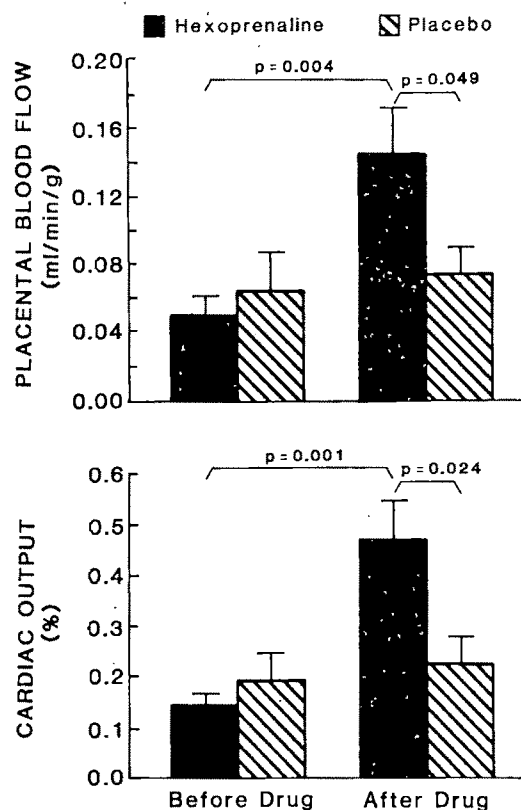


Fig. 2. Effect of hexoprenaline and placebo (saline solution) on maternal placental blood flow. Top panel expresses mean  $\pm$  SEM flow as milliliters per minute per gram. Lower panel is the mean  $\pm$  SEM percentage of total cardiac output distributed to the placentas.

spheres labeled with either gadolinium 153 or tin 113 (New England Nuclear, Boston, Massachusetts),  $15 \pm 3$   $\mu$ m in diameter, were sealed in a 15 cm segment of silicone rubber tubing. The microspheres were suspended in physiologic saline solution with Tween-80 (0.01%) added to prevent aggregation. The microsphere-containing catheter was wound around a wooden dowel rod to a height of 1.5 cm, placed in a gamma counting vial, and counted in a Model 1185 gamma well counter (Nuclear-Chicago, Des Plaines, Illinois) at the appropriate photopeak for the isotope used. After counting, the ends of the catheter were clipped and one end was attached to the left ventricular catheter while the other was attached to a syringe containing 0.5 ml of saline solution. The femoral reference blood sample collection was initiated at the rate of 0.5 ml/min, and after verification that it was withdrawing smoothly, the microspheres were flushed into the left ventricle with the 0.5 ml of saline solution during a period of 20 seconds. Collection of the arterial reference sample was continued for 1 minute after the end of the microsphere injection to ensure that all microspheres in transit in the arterial blood were collected.



**Table II.** Blood flow to the various organs

Organ	Before drug			After drug		
	Hexoprenaline	Saline solution	<i>p</i> Value	Hexoprenaline	Saline solution	<i>p</i> Value
Placenta	0.141 ± 0.066	0.193 ± 0.128	0.373	0.465 ± 0.197	0.224 ± 0.118	0.024
Uterus	1.268 ± 0.390	1.255 ± 0.670	0.967	1.343 ± 0.311	1.184 ± 0.259	0.341
Ovary	1.878 ± 0.643	1.173 ± 0.694	0.084	1.612 ± 0.543	1.115 ± 0.679	0.170
Kidney	14.007 ± 2.592	15.684 ± 4.981	0.452	10.899 ± 3.520	14.542 ± 3.231	0.080
Vagina	0.173 ± 0.083	0.192 ± 0.045	0.628	0.152 ± 0.062	0.197 ± 0.086	0.302
Spleen	1.738 ± 0.820	1.865 ± 0.910	0.796	1.318 ± 0.626	2.007 ± 0.725	0.093
Heart	4.351 ± 2.209	5.216 ± 1.917	0.471	4.839 ± 2.372	5.743 ± 1.819	0.463
Lungs	2.229 ± 1.255	2.106 ± 0.869	0.843	2.262 ± 1.332	1.739 ± 0.952	0.440

Values are mean ± SD.

The arterial reference blood sample and syringe washings were transferred to a plastic gamma counting tube. The femoral catheter was flushed with saline solution and reattached to the pressure transducer, and heart rate and arterial blood pressure were recorded again. Blood pressure after microsphere injection did not differ significantly from that measured before microsphere injection, indicating that this method does not significantly alter the cardiovascular physiologic characteristics.

The venous catheter was connected to an infusion pump and the awake animal was given a bolus injection of saline solution or hexoprenaline (0.5 µg/kg = 0.25 ml/kg). The length of the entire catheter had previously been measured and appropriate adjustments made for the bolus volume. The bolus injection was followed immediately by a 30-minute infusion of saline solution or active drug, administered at a dosage of 0.1 µg/kg/min. Heart rate and blood pressure were monitored throughout the saline solution or drug administrations. Upon completion of the infusion, the alternate set of microspheres was injected and a second arterial reference sample drawn. Each animal was then killed with an overdose of sodium pentobarbital. The entire length of ventricular catheter and silicone rubber tubing was wound around the dowel rod again to a height of 1.5 cm, inserted into the gamma counting tube, and recounted in the gamma well counter. The counts per minute for the calculation of cardiac output were determined as the difference between the counts per minute of the microsphere catheter before injection and the counts per minute of the entire catheter after injection. Seven rats received hexoprenaline and six the saline solution.

After the rat was weighed, the uterus was removed and the fetuses and placentas were carefully detached from the uterine wall. The fetuses and placentas were separated from the amniotic fluid and membranes, gently blotted dry, and weighed separately; the placentas were placed in gamma counting tubes, three to four placentas per tube. The uterus was separated into right

and left horns and placed in separate gamma counting tubes. The other organs and tissues were also placed in counting tubes to ensure that the levels of all tissues were kept below 1.5 cm to avoid loss of counting efficiency. The tissues and the arterial reference were counted in the gamma well counter, and the cardiac output and organ distribution of cardiac output were calculated as follows, where output and flow are measured in milliliters per minute and cpm is counts per minute:

$$\text{Cardiac output} = \frac{\text{cpm injected} \times \text{arterial reference flow rate}}{\text{cpm in arterial reference}}$$

$$\text{Organ blood flow (\% cardiac output)} = \frac{\text{cpm in organ}}{\text{cpm injected}} \times 100$$

$$\text{Organ blood flow} = \frac{\text{cpm in organ} \times \text{arterial reference flow rate}}{\text{cpm in arterial reference}}$$

In a preliminary experiment the whole animal was also cut up into pieces, which were placed in individual gamma counting vials (maximum tissue height in each vial = 1.5 cm) for gamma counting. The counts per minute injected in this case were calculated as the sum of the counts per minute of the whole animal. There was close agreement (<3% difference) in the counts per minute injected between this method and that of counting the counts per minute in the catheter before and after injection. Therefore, the differential counting of the catheter method was used in all subsequent experiments for convenience.

The statistical tests used were the Student *t* test for between-group comparisons and the paired *t* test to assess changes within the same groups.

## Results

There were no significant differences in maternal weight, fetal weight, or litter size between the hexoprenaline and saline solution groups of rats (Table I). Cardiac output, heart rate, and blood pressure before

Organ	Mean difference			Hexoprenaline changes, paired t test (p value)
	Hexoprenaline	Saline solution	p Value	
Placenta	0.324 ± 0.144	0.032 ± 0.205	0.012	0.001
Uterus	0.076 ± 0.358	-0.071 ± 0.600	0.596	0.597
Ovary	-0.265 ± 0.718	-0.058 ± 0.555	0.578	0.366
Kidney	-3.107 ± 2.337	-1.142 ± 3.019	0.212	0.013
Vagina	-0.021 ± 0.068	0.005 ± 0.097	0.585	0.448
Spleen	-0.420 ± 0.776	0.142 ± 0.689	0.199	0.202
Heart	0.488 ± 1.474	0.527 ± 1.477	0.976	0.419
Lungs	0.033 ± 1.792	-0.367 ± 0.995	0.638	0.085

drug administration did not differ significantly between the two groups.

The mean ( $\pm$ SD) total cardiac output values after hexoprenaline and saline solution administration were  $30.9 \pm 6.89$  and  $30.1 \pm 4.66$  ml/min/100 gm, respectively. Hexoprenaline increased the mean heart rate and decreased the blood pressure (Fig. 1). Maternal placental blood flow was significantly increased (198%), from  $0.141 \pm 0.066$  to  $0.465 \pm 0.197$  ml/min/gm after hexoprenaline administration (Table II, Fig. 2). Blood flow to the kidneys significantly decreased (24%), from  $14.0 \pm 2.6$  to  $10.9 \pm 3.5$  ml/min/gm after hexoprenaline (Table III). The blood flows to the other organs were not affected by hexoprenaline. Administration of saline solution did not produce significant changes in the blood flows to any of the organs.

### Comment

Compared with saline solution, hexoprenaline significantly increased blood flow to the placentas of pregnant rats on day 14 of gestation by increasing the fraction of cardiac output distributed to the placentas. This suggests vasodilation of the uteroplacental vasculature. In another study, with the diet-restricted pregnant rat model, long-term oral administration of hexoprenaline from day 5 of gestation resulted in a significant increase in placental blood flow measured on day 21 of gestation.<sup>1</sup>

In pregnancy  $\alpha$ -adrenergic stimulation produces vasoconstriction and decreased blood flow in the uteroplacental circulation.<sup>5</sup> The vasculature of the nonpregnant uterus responds to  $\beta$ -adrenergic stimulation with vasodilation.<sup>6</sup> In pregnancy, however, the results of studies on  $\beta$ -adrenergic agonists are confusing and seem to depend on the specific drug, the species studied, and the experimental methodology. In near-term pregnant ewes, ritodrine, isoxsuprine, salbutamol, and terbutaline caused an increase in uterine vascular resistance and a decrease in uteroplacental blood flow.<sup>7-9</sup> Isoxsuprine had a greater effect than salbutamol or terbutaline. Fenoterol, on the other hand, caused a small reduction in uterine vascular resistance and an

11% increase in uterine blood flow.<sup>9</sup> In radioangiographic studies in near-term anaesthetized pregnant rhesus monkeys, Wallenburg et al.<sup>10</sup> found an increase in placental blood flow after administration of metaproterenol.

Studies in humans are encumbered by the necessity of having to use various techniques that may only indirectly reflect uteroplacental blood flow. Use of indium In 113m and gamma counting showed that fenoterol increased uteroplacental blood flow in the laboring patient<sup>11</sup> and that salbutamol decreased blood flow in the absence of uterine contractions.<sup>12</sup> With the use of xenon 133, fenoterol and isoxsuprine were shown to increase myometrial blood flow in nonlaboring women, but the intervillous blood flow remained unchanged.<sup>13</sup> With the use of a thermistor probe in the anterior lip of the cervix, ritodrine produced no change in blood flow in normal pregnancies, but in pregnancies with hypertension and intrauterine growth retardation, blood flow was significantly increased when compared with that after placebo.<sup>14</sup> Studies in the isolated human placenta showed that terbutaline did not change basal vascular resistance, but when the placental vessels were constricted with angiotensin, terbutaline produced a decrease in vascular resistance.<sup>15</sup>

Our finding that renal blood flow is significantly reduced after an intravenous infusion of hexoprenaline agrees with the results of Kleinman et al.,<sup>16</sup> who demonstrated a 70% reduction in fractional distribution of cardiac output to the kidney after an infusion of ritodrine in the pregnant ewe. These authors observed that the decrease in renal plasma flow was most likely due to active vasoconstriction, which may be mediated through an increase in renin-angiotensin levels. Available information appears to implicate circulatory overload as the primary factor in the cause of pulmonary edema produced by  $\beta$ -adrenergic receptor stimulants.

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## Effect of long-term administration of $\beta_2$ -sympathomimetic drug in the diet-restricted pregnant rat model

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To assess whether the maternal-fetal balance could be altered in favor of the fetus during malnutrition by increasing uteroplacental blood flow, 0.5 mg of hexoprenaline per day was added to the diet of one group of diet-restricted rats, while another group served as controls. The radionuclide-labeled microsphere method was used to determine blood flow to the maternal placenta and other organs. Maternal carcass weight but not fetal or placental weights were increased in the hexoprenaline-fed rats. Blood flow to the ileum, jejunum, hepatic artery, kidneys, and placenta was significantly greater in the hexoprenaline group compared with those rats fed the restricted diet alone. Although the placental blood flow was increased in the hexoprenaline-fed rats, the supply of nutrients remained restricted, and in the mother the inherent maternal-fetal balance was maintained by an increase in the blood flow to the liver and small intestine. (*AM J OBSTET GYNECOL* 1986;154:314-7.)

**Key words:** Dietary restriction, placental blood flow, hexoprenaline,  $\beta$ -sympathomimetic drug

Uteroplacental blood flow increases dramatically during pregnancy to support the nutritional demands

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of the rapidly growing fetus. This is made possible by an increase in maternal plasma volume and cardiac output, a decrease in vascular resistance, and an increase in the fractional distribution of cardiac output to the uterus.<sup>1,2</sup>

A 50% dietary restriction in the rat from day 5 of pregnancy until term results in an absence of maternal weight gain beyond that of the conceptus.<sup>3,4</sup> At term, the diet-restricted rats were significantly smaller than the pregnant rats fed ad libitum, which was reflected

by significantly lower weights for most body organs. Growth of the fetuses was also retarded, resulting in a 17% lower mean fetal weight and a 26% lower mean placental weight at term. Furthermore, the dietary restriction caused a 40% to 50% reduction in uteroplacental blood flow at term,<sup>2,3</sup> which resulted from a decrease in cardiac output and a decreased fraction of the cardiac output delivered to the uterus.<sup>2</sup> The percentage of cardiac output distributed to the hepatoportal circulation is increased, while that to other organs is maintained.<sup>4</sup> The reduction in uteroplacental blood flow appears to be mediated by  $\alpha$ -adrenergic stimulation.<sup>6</sup> Thus the mammalian fetus is not a metabolic parasite, and during periods of malnutrition the cardiac output in the mother was redistributed to maximize the mother's own use of available nutrients and to protect her against severe depletion of nutrient stores by the developing fetus.

We have recently demonstrated that hexoprenaline (Byk Gulden, Germany), a  $\beta_2$ -sympathomimetic drug, is able to significantly increase uteroplacental blood flow in the pregnant rat at day 14 of gestation.<sup>7</sup> To further explore the maternal-fetal interactions during malnutrition and to assess whether any fetal benefits would accrue, hexoprenaline was added to the diet of the malnourished pregnant rat model.

#### Material and methods

Timed pregnant Sprague-Dawley rats were obtained from Zivic-Miller Laboratories Inc., Allison Park, Pennsylvania, and were housed individually in stainless steel wire-bottom cages. The temperature of the room was maintained at 25°C, with a 12-hour light–12-hour dark cycle with the lights on at 6:00 AM. The animals were fed a sprayed egg white test diet (Teklad Mills, Madison, Wisconsin) and drinking water containing 13 mg/L of zinc ad libitum until day 5 of gestation. Specific details of the diet composition were described previously.<sup>2</sup> On day 5 of gestation, the pregnant rats were divided into two groups. One group (controls,  $n = 10$ ) was fed a restricted diet of 8 gm/day from day 5 through day 9 and 11 gm/day from day 10 through day 21; group 2 (treatment,  $n = 12$ ) was fed the same restricted diet as the control group with the addition of 0.5 mg of hexoprenaline per day. Feeding from day 5 through day 21 took place twice per day with half the daily ration being given in the morning (9:00 AM to 10:00 AM) and the remaining half being given in the evening (4:00 PM to 5:00 PM).

On day 21 of gestation the animals were anesthetized with sodium pentobarbital (30 mg/kg, intraperitoneal administration) and prepared for measurement of cardiac output and organ distribution of blood flow as described previously.<sup>7,8</sup> Polyethylene catheters were inserted into the left ventricle of the heart via the right carotid artery and into the left femoral artery. Place-

**Table I.** Total body, fetal, and placental weights at term

Determination	Control group	Hexoprenaline group	<i>p</i> Value
Total weight (gm)	292.20 $\pm$ 5.06	308.58 $\pm$ 2.85	0.008
Carcass weight (gm)	183.90 $\pm$ 3.30	197.36 $\pm$ 1.66	0.003
Fetal weight (gm)	4.80 $\pm$ 0.10	4.56 $\pm$ 0.11	0.12
Placental weight (gm)	0.50 $\pm$ 0.02	0.49 $\pm$ 0.02	0.54
Litter size	10.50 $\pm$ 0.79	11.67 $\pm$ 0.61	0.25

Values are means  $\pm$  SEM.

ment of the catheter tip in the left ventricle was confirmed by the left ventricular pressure pulse tracing, and the exact position was verified at autopsy. Both catheters were filled with heparinized saline solution (20 IU/ml) to prevent clotting and the femoral arterial catheter was connected to a Statham P23Gb pressure transducer and arterial pressure was recorded on a Gould 2200S recorder. After 15 to 20 minutes, when heart rate and blood pressure were stable, cardiac output and organ blood flow were determined in the anesthetized rat by the reference sample microsphere technique.<sup>9</sup>

Radionuclide-labeled microspheres (15  $\pm$  3  $\mu$ m in diameter), suspended in physiologic saline solution with Tween-80 (0.01%) added to prevent aggregation, were used for measurement of cardiac output and its tissue distribution. Approximately 100,000 microspheres labeled with tin 113 or gadolinium 153 were sealed in a 15 cm length of silicone rubber tubing and wound around a wooden dowel rod to a height of 1.5 cm. The rod was placed in a gamma counting vial and counted in the gamma well counter at the appropriate photopeak for the isotope used. After counting, the ends of the microsphere-containing catheter were clipped and one end was attached to the ventricular catheter while the other was attached to a syringe containing 0.5 ml of saline solution. The microspheres were flushed into the left ventricle during a 20-second period while an arterial blood reference sample was simultaneously withdrawn at the rate of 0.5 ml/min. The dam was then killed with an overdose of sodium pentobarbital. The microsphere injection catheter was wound around the dowel rod again to a height of 1.5 cm, inserted into the gamma counting tube, and recounted in the gamma well counter. For the calculation of cardiac output the counts per minute were determined as the difference between the counts per minute of the microsphere catheter before injection and the counts per minute of the entire catheter after injection.

After the rat was weighed, the fetuses and placentas were carefully detached from the uterine wall, separated from the amniotic fluid and membranes, gently blotted dry, and weighed separately. The uterus was separated into right and left horns and counted in separate gamma counting tubes. The remaining carcass



**Table II.** Hemodynamic measurements

Determination	Control group	Hexoprenaline group	p Value
Cardiac output (ml/min/100 gm)	15.76 ± 1.42	18.57 ± 2.15	0.31
Mean blood pressure (mm Hg)	84.60 ± 6.75	93.67 ± 4.25	0.25
Systolic pressure (mm Hg)	104.00 ± 9.70	115.33 ± 5.88	0.31
Diastolic pressure (mm Hg)	68.33 ± 6.20	74.00 ± 4.38	0.45
Heart rate (bpm)	332.40 ± 9.47	353.00 ± 9.96	0.15

Values are means ± SEM.

was weighed for determination of net maternal weight and the remaining organs were removed, weighed, and placed in separate gamma counting tubes. The height of all tissues in the vials were kept below 1.5 cm to avoid a loss of gamma counting efficiency. Cardiac output and tissue distribution of blood flow were calculated as follows, where output and flow are measured in milliliters per minute and cpm is counts per minute:

$$\text{Cardiac output} = \frac{\text{cpm injected} \times \text{arterial reference flow}}{\text{cpm in arterial reference}}$$

$$\text{Blood flow} = \frac{\text{cpm in organ} \times \text{arterial reference flow}}{\text{cpm in arterial reference}}$$

Data analysis was carried out with the use of the statistical computer program BMDP3D.<sup>10</sup> This program tests for equality of variances between groups by an F test and then compares two group means by two-sample *t* tests with and without the assumption of the equality of variances.

### Results

There was no difference in mean maternal weight between the two groups on day 5 of gestation. The hexoprenaline group weighed 271.2 ± 4.65 gm and the control group weighed 265.1 ± 4.46 gm. By day 21 of gestation the hexoprenaline-fed rats were significantly heavier than the controls (Table I). This was due entirely to an increase in carcass weight as neither fetal nor placental weights or litter size differed significantly between the two groups. There was no difference in any of the maternal organ weights between the two groups, except in the ovaries, which were heavier in the hexoprenaline group (0.11 ± 0.006 vs. 0.09 ± 0.006 gm, *p* = 0.03).

Cardiac output, blood pressure, and heart rate did not differ between the two groups at term (Table II). The blood flows to the various organs are listed in Table III. Compared with controls, the hexoprenaline-fed rats had significantly increased blood flow to the ileum (70%), jejunum (58%), hepatic artery (39%), kidneys

**Table III.** Organ blood flow measurements (milliliters per minute per 100 gm)

Organ	Control group	Hexoprenaline group	p Value
Heart	525.48 ± 58.51	619.03 ± 136.06	0.56
Lungs	110.07 ± 17.19	145.04 ± 34.57	0.40
Kidney	247.91 ± 19.19	343.87 ± 34.99	0.03*
Adrenal	411.64 ± 51.23	444.50 ± 49.69	0.65
Hepatic artery	29.80 ± 2.24	41.32 ± 4.66	0.04*
Spleen	68.62 ± 18.52	94.47 ± 34.37	0.54
Pancreas	54.94 ± 10.67	80.12 ± 10.52	0.11
Stomach	41.70 ± 8.28	60.39 ± 6.76	0.09
Duodenum	178.33 ± 14.07	251.38 ± 38.44	0.11
Jejunum	149.24 ± 17.21	235.76 ± 29.83	0.03*
Ileum	138.37 ± 20.50	237.36 ± 32.31	0.02*
Cecum	116.78 ± 6.82	133.90 ± 18.62	0.43
Colon	42.15 ± 4.54	63.47 ± 10.45	0.10
Muscle	14.86 ± 4.58	19.75 ± 2.75	0.35
Ovaries	662.95 ± 136.95	816.01 ± 141.90	0.45
Uterus	15.21 ± 1.65	17.90 ± 1.71	0.28
Placentas	57.18 ± 4.24	74.18 ± 4.97	0.02*
Vagina	29.46 ± 4.15	39.45 ± 6.67	0.24

Values are means ± SEM.

\*Significant difference.

(39%), and placentas (30%). The increased blood flow to the stomach in the hexoprenaline group almost reached statistical significance (*p* = 0.09). Blood flow to the heart, lungs, pancreas, ovaries, or myometrium was not increased.

### Comment

In diet-restricted rats, the addition of 0.5 mg of hexoprenaline to the diet resulted in an increased maternal blood flow to the placentas, kidneys, hepatic artery, jejunum, and ileum. Although the fetal and placental weights did not differ in the two groups, the maternal carcass weight was significantly greater in the hexoprenaline group. We have previously shown that, compared with rats fed ad libitum, diet-restricted pregnant rats demonstrate an increase in blood flow per unit weight and percentage cardiac output to the hepatic-portal circulation.<sup>4</sup> This is probably a protective mechanism to ensure a continued supply of glucose when exogenous dietary supplies are lacking. Increased hepatic gluconeogenesis has been observed in fasting pregnant rats<sup>11</sup> and sheep.<sup>12</sup>

During periods of nutritional deprivation, there appears to be a delicate balance between the needs of the mother and those of the fetus. In the malnourished rats there was a reduction in uteroplacental blood flow with concomitant fetal growth retardation, which may be beneficial for fetal survival under these circumstances. In the pregnant ewe, a decrease in maternal placental blood flow produced a rapid decrease in umbilical blood flow<sup>13</sup> and was associated with decreased fetal substrate uptake and oxidative metabolism.<sup>14</sup> The reduction in substrate demand by the fetus benefits the mother by limiting the drain of nutrients needed for

maintenance of her own metabolic rate and thus survival and at the same time ensures fetal survival. When diet-restricted rats were provided with adequate food during the last week of gestation, uteroplacental blood flow and fetal weight were the same as controls fed ad libitum by day 21 of gestation, although maternal weight remained significantly less.<sup>2</sup> Thus, when an adequate supply of nutrients was made available to the mother, the fetus appeared to have preferential access.

Although the placental blood flow was increased in the hexoprenaline-fed rats, the supply of nutrients remained restricted and the hepatoportal (liver and small intestine) blood flow and the carcass weight increased in the mother. It is theorized that if the supply of nutrients were gradually increased, fetal weight gain would also occur, in keeping with the inherent maternal-fetal balance. Thus the administration of a  $\beta$ -adrenergic receptor agonist, in the presence of a 50% reduction in protein-calorie intake, is not able to produce preferential channeling of nutrients to the fetus by increasing placental blood flow without affecting other maternal vascular beds at the same time.

The increased uteroplacental circulation in pregnancy may be partially dependent on adequate estrogen production. While no data are available for the rat, estrogens are decreased during maternal malnutrition in humans, and dietary supplementation during the last 4 weeks of pregnancy increases urinary estrogen excretion.<sup>15</sup> The maternal ovaries were the only organs that were heavier in the hexoprenaline-fed rats.

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# The effect of maternal consumption of alcohol on human umbilical artery blood flow

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A Doppler ultrasound technique was used to measure the effect on human umbilical artery impedance of a single moderate dose of alcohol consumed by mothers compared to a control solution in six normal third-trimester pregnancies. No significant difference in impedance was detected between the alcohol and the control solutions during the subsequent 90 minutes. The results suggest that the known toxicity of maternally ingested alcohol is probably mediated by some mechanism other than a significant acute alteration in fetoplacental blood flow characteristics. (*Am J Obstet Gynecol* 1986;154:318-21.)

**Key words:** Alcohol, maternal consumption, umbilical artery impedance

Chronic maternal consumption of alcohol is associated with well-documented detrimental effects on the fetus<sup>1</sup> although the mechanism of action is not clear. Single doses of alcohol also have been shown to have an acute effect on the fetus. McLeod et al.<sup>2</sup> have shown in human studies that a single moderate dose of alcohol (0.25 gm/kg) virtually abolishes fetal breathing movements. Mukherjee and Hodgen<sup>3</sup> in a study of the effects of maternal administration of alcohol on the fetal monkey used a larger dose (3.0 gm/kg of maternal weight) and noticed incidentally that the umbilical cord lost its turgidity, which suggested that a marked decrease in fetoplacental vascular impedance occurred.

Pulsed Doppler ultrasound provides a noninvasive means of measuring the impedance to flow within deeply situated blood vessels<sup>4</sup> including the human umbilical artery.<sup>5</sup> This technique was used to investigate the acute effect on human umbilical artery impedance of drinking alcohol in the same quantity as that known to abolish fetal breathing movements.<sup>2</sup>

## Material and methods

Six pregnant women between 34 and 36 weeks of amenorrhea (mean = 35 weeks and 2 days) who gave verbal informed consent made up the study group. Each patient was asked to drink either a solution of alcohol in soda water or soda water alone in a randomized fashion on each of two successive days. All were

social drinkers who had taken alcohol during this pregnancy at some time but consumed less than 40 gm of alcohol per week (equivalent to less than four glasses of wine). All six mothers had fetal maturity confirmed ultrasonically at 16 weeks of amenorrhea, and all were subsequently delivered normally of singleton infants of appropriate weight after 38 weeks' gestation.

No alcohol was consumed during the 24 hours preceding the 2-day study period. All mothers had eaten a normal light breakfast and light lunch and had similar meals on each of the two days. The study sessions started at 4 PM each day, after the subjects had fasted from 1 PM.

A solution of ethanol (0.25 gm/kg of maternal weight) in soda water as a 15% solution was used, and the control dose was an equal volume of soda water alone.<sup>2</sup> To standardize maternal ingestion times, the solution was divided into three equal aliquots each to be taken by the patient over successive 5-minute periods. Thus the total time to consume the test solution was 15 minutes. The solutions were prepared and randomized by a third party not otherwise involved in the study.

The alcohol solution comprised  $0.317 \times$  (maternal weight in kilograms) ml of 100% pure dehydrated ethanol mixed with  $1.79 \times$  (maternal weight in kilograms) ml of soda water, whereas the control solution comprised  $2.107 \times$  (maternal weight in kilograms) ml of soda water alone. The pure alcohol had a specific gravity of between 0.7904 and 0.7935 with 1 ml being equivalent to 0.79 gm of ethyl alcohol. Thus a 70 kg patient would receive 17.5 gm of ethanol or 22.2 ml of pure alcohol solution mixed with 125 ml soda water. This is equivalent to approximately 1.75 standard measures of 40% spirit.

Seven 2 ml aliquots of maternal venous blood for estimations of serum ethanol were taken on each day of the study at the following times: zero time minus 15

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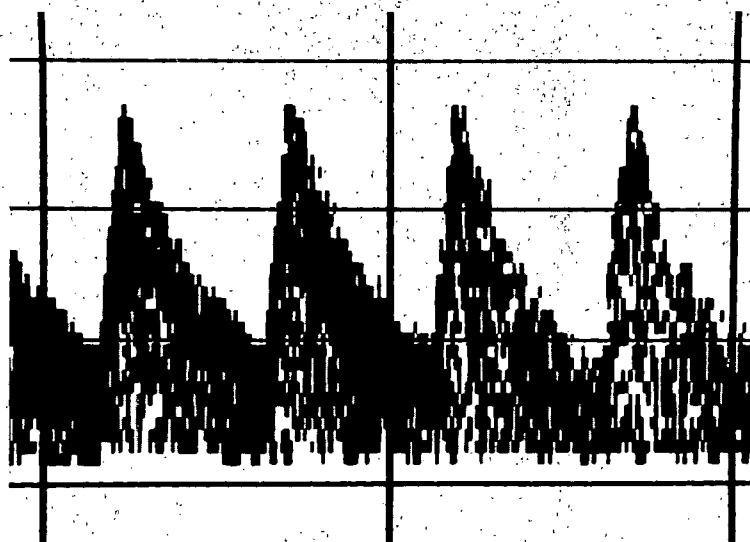


Fig. 1. Umbilical artery sonograms obtained from a normal fetus of 36 weeks' gestation representing red blood cell velocities (vertical axis) with time.

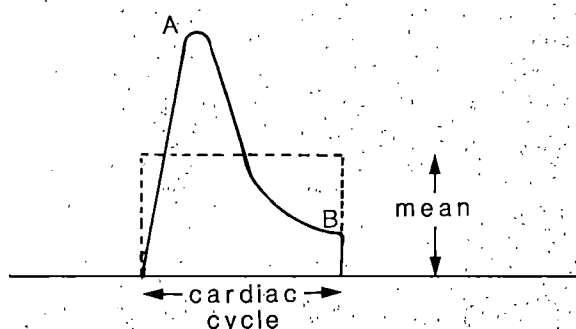


Fig. 2. Measurements required from the maximum frequency outline of umbilical artery sonograms to calculate the pulsatility index.

minutes, zero time plus 15, 30, 45, 75, and 105 minutes. The test solution was consumed during the 15 minutes after zero time.

A pulsed Doppler Duplex ultrasonic sector scanning system (Advanced Technology Laboratories, Mark V) with a 3 MHz probe was used. This equipment presents two-dimensional real-time imaging together with pulsed Doppler ultrasound in a quasi-simultaneous format. The Doppler-shifted frequencies resulting from movement occurring within the sample volume (4 mm × 2 mm) are displayed visually against time as time-velocity waveforms or sonograms (Fig. 1); since the shifted frequencies resulting from blood flow are within the audible range, these signals are also passed via an amplifier to an audio output. The sample volume of the Doppler system can be moved to monitor the luminal center of the umbilical artery where maximal blood velocity occurs<sup>6</sup> by listening to the audio output in a manner akin to tuning a radio receiver. The sonograms obtained are preserved by a line scan re-

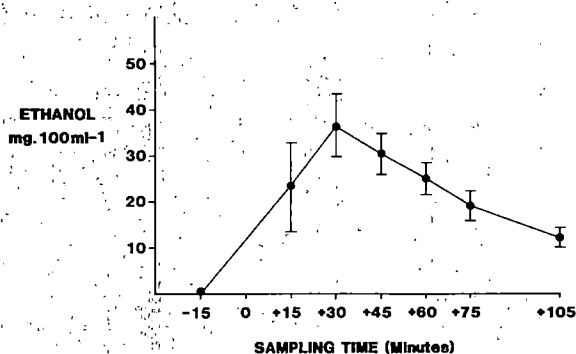


Fig. 3. Mean maternal venous blood alcohol levels ( $\pm$ SE) on the alcohol-dose days (mg/100 ml).

corder. Umbilical artery impedance was estimated by calculation of the pulsatility index, which has been shown previously to be sensitive to changes in vascular resistance.<sup>7</sup> The measurements required (A-B/mean) to calculate the pulsatility index<sup>8,9</sup> are shown in Fig. 2. The mean value for the pulsatility index of each of 10 consecutive time-velocity waveforms was obtained from the umbilical artery at zero time minus 15 minutes and at zero time plus 30, 60, and 105 minutes. The sonographic printouts from the Doppler equipment were labeled with random numbers and subjected to secondary waveform analysis several days later with use of the digitizing tablet of a minicomputer (Kontron, Cardio 80).

### Results

No ethanol was detected in the maternal blood samples taken on any of the days the control dose was given. On the days the alcohol dose was given the mean peak ethanol concentration ( $\pm$ SE) was  $36.8 \pm 6.8$  mg/100



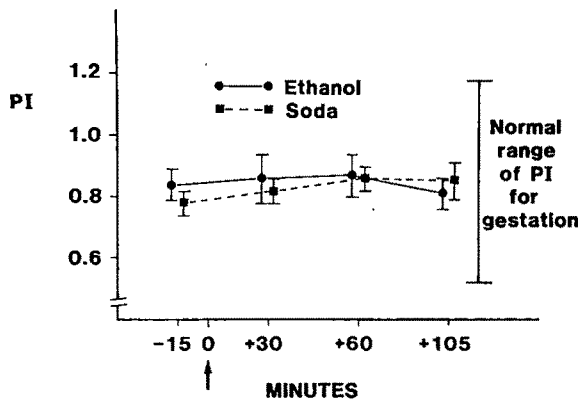


Fig. 4. Mean values ( $\pm$ SE) for umbilical artery pulsatility index (PI) for each scanning session on both the alcohol-dose and control-dose days.

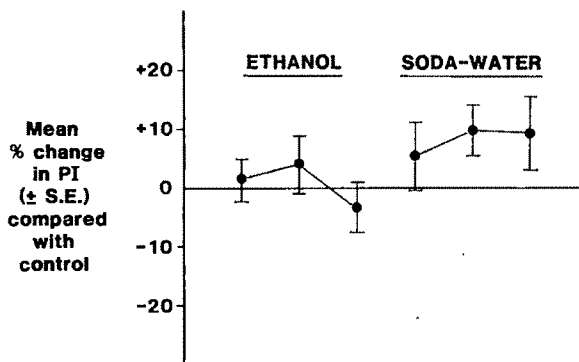


Fig. 5. Mean percentage change ( $\pm$ SE) in umbilical artery pulsatility index (PI) at plus 30, 60, and 105 minutes after zero time when compared to the control value at zero time minus 15 minutes for both study days.

ml and was obtained at zero time plus 30 minutes (Fig. 3).

The mean values of the pulsatility index as calculated from 10 consecutive umbilical artery sonograms at each observation time are listed in Table I and shown graphically in Fig. 4. The percentage change in pulsatility index observed at each of the three postingestion observation periods when compared to the control value at zero minus 15 minutes is shown in Fig. 5. There was no significant difference in the mean pulsatility index values at each observation time between the days alcohol was given and the control days. The percentage change in pulsatility index between the first and last measurement was greater on the days alcohol was given than on the control days, but this did not reach statistical significance ( $p > 0.1$ ;  $r = -0.15$ ). Also the percentage change in pulsatility index between the first and last scans on the alcohol days for each subject was compared to that individual's maximum serum alcohol concentration, but no consistent relationship was found ( $p > 0.1$ ;  $r = -0.11$ ).

All values for the pulsatility index throughout this

Table I. Mean values for umbilical artery pulsatility index obtained at each of the four examination times on each of the two study days (see also Fig. 4)

Time (min)	Mean pulsatility index	SE
Alcohol day		
Zero - 15	0.84	0.05
Zero + 30	0.86	0.08
Zero + 60	0.87	0.07
Zero + 105	0.81	0.05
Control day		
Zero - 15	0.78	0.04
Zero + 30	0.82	0.04
Zero + 60	0.86	0.04
Zero + 105	0.85	0.06

study remained within the normal range previously established in this population of normal pregnant women.<sup>10</sup>

#### Comment

The mean peak ethanol concentration ( $\pm$ SE) was 36.8 ( $\pm$ 6.8) mg/100 ml, which is comparable to that found by McLeod et al.,<sup>2</sup> namely, 33 ( $\pm$ 3.7) mg/100 ml. Peak levels in both studies occurred at zero time plus 30 minutes. Although this maternal alcohol concentration is known to affect the fetus directly by abolishing fetal breathing movements, it did not significantly alter umbilical artery impedance as measured by the pulsatility index.

Brien et al.<sup>11</sup> in a study of amniotic fluid levels of alcohol after maternal consumption (0.3 gm/kg of maternal weight) in patients undergoing second-trimester terminations of pregnancy showed that the peak amniotic fluid level of alcohol lagged behind peak maternal venous levels by some 90 minutes; although no alcohol was detectable in maternal venous blood after 3.5 hours, significant levels were still present in the amniotic fluid at this time. It is therefore possible that two to 3 hours after maternal ingestion of a dose of alcohol the fetus is still being affected by the drug, since the fetus continually swallows amniotic fluid. This hypothesis is somewhat supported by the fact that placental resistance as measured by the mean pulsatility index tended to decrease between time zero and time zero plus 105 minutes after alcohol ingestion (Fig. 5), whereas it increased on the control days although this difference was not statistically significant. Nevertheless no acute effects were observed at a time when fetal alcohol levels might be expected to be maximal, and all pulsatility index values remained within the previously established normal range.<sup>10</sup>

It is concluded from this study that a moderate dose of alcohol ingested by a mother during the normal third trimester does not significantly alter impedance to flow

within the umbilical artery as measured by the Doppler technique. The known toxicity of drinking alcohol therefore may well be mediated by some other mechanism and not by acute changes in fetoplacental blood flow characteristics.

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## The effect of electrical stimulation on behavioral states in the fetal lamb

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Brachial nerve stimulation at two frequencies (0.01 and 0.05 pulse per second, pps) with the fetus in either low-voltage electrocortical activity with eye movements or high-voltage electrocortical activity without eye movements was studied in five chronically catheterized fetal lambs at 130 to 140 days' gestation. During low-voltage electrocortical activity with eye movements, there were slight alterations in electrocortical state and electromyographic activity at 0.01 pps. At 0.05 pps these electromyographic changes were enhanced above those expected as a result of state changes but returned to control values during the recovery period. During high-voltage electrocortical activity without eye movements, the state changes were much more dramatic, while electromyographic activity increased significantly only at 0.05 pps. It was of note that in both high- and low-voltage electrocortical activity the fetal heart rate changed only during the recovery period at 0.05 pps. It is concluded that fetal behavioral state influences the interpretation of biophysical measurements in the fetus and that the effect of stimulation is more pronounced if applied when the fetus is in high-voltage electrocortical activity without eye movements. (*AM J OBSTET GYNECOL* 1986;154:321-8.)

**Key words:** Stimulation, behavioral states, electrocortical activity

In clinical obstetrics, behavioral states in the fetus, as defined by fetal heart rate and its variations,<sup>1,2</sup> fetal breathing, and gross fetal body movements,<sup>3,4</sup> provide

the physiologic basis of the nonstress test as an important evaluator of fetal health in the antenatal period.<sup>5</sup> However, since periods of rest are separated by periods of activity under normal conditions, various investigators have undertaken to stimulate the fetus during periods of flat fetal heart rate tracings in an attempt to identify the sick fetus.<sup>6-9</sup>

Few attempts, however, have been made to try to clarify the physiologic characteristics underlying such stimulation maneuvers.<sup>10-12</sup>

The purpose of the present set of experiments was to test the hypothesis that clinically measurable param-

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**Table I.** Stimulation experiments: low-voltage electrocortical activity, stimulation rate = 0.01 pps

	Control	Stimulation	Recovery
Electrocortical activity (%)			
High	0.0	3.5 ± 1.1	28.7 ± 2.7*
Low	100.0	89.6 ± 2.0†	60.9 ± 2.4*
Transitional	0.0	6.9 ± 1.3	10.4 ± 1.0‡
Eye movements present (%)	95.7 ± 2.1	79.1 ± 2.7*	50.9 ± 3.3*
Electromyographic activity			
Nuchal	1.37 ± 0.13	1.49 ± 0.09	1.16 ± 0.08
Biceps	0.84 ± 0.10	1.09 ± 0.07‡	0.79 ± 0.07
Triceps	1.08 ± 0.13	1.48 ± 0.09‡	1.09 ± 0.07
Tracheal pressure (%)	80.4 ± 4.1	70.0 ± 3.0	57.0 ± 3.3*
Blood pressure (mm Hg)	37.7 ± 0.7	38.5 ± 0.5	38.1 ± 0.4
Fetal heart rate (bpm)	144.7 ± 1.5	148.2 ± 0.9‡	152.7 ± 1.1*
PaO <sub>2</sub> (mm Hg)	21.4 ± 0.3	21.2 ± 0.2	20.8 ± 0.2

Electrocortical activity is represented as the percentage of time spent in each state. Eye movements and tracheal pressure are represented as the percentage of time during which rapid eye movements and fetal breathing were present. Electromyographic activity is represented as the mean number of electromyographic bursts occurring per 0.5-minute epoch. All values are represented as mean ± SEM for each consecutive 0.5-minute epoch analyzed (n = 23).

\*p < 0.001.

†p < 0.01.

‡p < 0.05.

eters, such as fetal heart rate, fetal breathing, and gross fetal body movements, are affected differently when stimulation of the chronically catheterized fetal lamb is carried out with the fetus in different behavioral states, as defined by electrocortical activity and the presence or absence of rapid eye movements.

### Methods

Stimulation experiments were performed on fetuses of five pregnant ewes between 130 and 140 days' gestation. Fetuses were operated on at 120 days' gestation. Paired stainless steel electrodes were implanted bilaterally on the parietal dura, at the inner and outer canthus of one eye, in the nuchal muscles, in the muscles of the biceps and triceps of one forelimb, and into the nerve sheath of the brachial nerve of the same forelimb. All recording electrodes were referred to a single electrode implanted on the fetal scalp. Polyvinylchloride catheters were implanted into the fetal jugular vein, fetal trachea, and fetal carotid artery. An open-ended catheter was placed into the amniotic fluid to record amniotic pressures. Four fetuses also had an intravascular PO<sub>2</sub> electrode (Kontron-Roche, Basel, Switzerland) inserted through the fetal carotid catheter, which rested at the level of the aorta. Three silver-silver chloride electrodes were implanted subcutaneously on the chest wall to record the fetal electrocardiogram. In addition, catheters were inserted into the maternal femoral artery and vein for sampling.

After operation the ewes were given Demerol intramuscularly to control pain for 2 days and antibiotics for 3 days. Fetuses were also given penicillin G intravenously and in the amniotic fluid for 3 days after operation. The animals were housed in metabolic cages

and allowed free access to food and water. Experiments began after the fourth postoperative day.

Biophysical data were displayed on a Grass Model 7C polygraph (Grass Instruments, Quincy, Massachusetts). Continuous recordings were obtained of electrocortical activity, eye movements, nuchal electromyograph, biceps electromyograph, triceps electromyograph, fetal heart rate, intravascular PaO<sub>2</sub>, tracheal pressure, and blood pressure corrected for amniotic fluid pressure.

Stimulation experiments were performed with the use of a Grass S10SCM Stimulator with Stimulus Isolation Unit SIU8TA (Grass Instrument Company, Quincy, Massachusetts). The electrodes implanted in the brachial nerve were connected to the stimulus isolation unit, and stimuli were applied to the brachial nerve at 50 V for a duration of 1 ms and a delay of 10 ms. A dose-response curve was established with one sheep by an alteration in stimulation rate, and in subsequent experiments stimulation rates of 0.01 and 0.05 pulse per second (pps) were used.

Previous experiments in our laboratory have established an average duration of 13.9 ± 0.5 minutes in low-voltage electrocortical activity and 9.4 ± 1.0 minutes in high-voltage electrocortical activity.<sup>13</sup> Therefore, to obtain a meaningful control period and an adequate stimulation and recovery period, we felt it appropriate to limit the experiment to 12 minutes. Thus experiments were designed to consist of a 2-minute control period, a 5-minute stimulation period, and a 5-minute recovery period. The criteria for the control phase of the experiments were based on electrocortical activity. A maximum of eight experiments per day were performed in each preparation. The experiments began when the fetus was either in true high-voltage electro-

**Table II.** Stimulation experiments: low-voltage electrocortical activity, stimulation rate = 0.05 pps

	Control	Stimulation	Recovery
Electrocortical activity (%)			
High	0.0	0.0	21.1 ± 2.2*
Low	100.0	94.7 ± 2.8	56.3 ± 1.9*
Transitional	0.0	5.3 ± 2.8	22.6 ± 2.4*
Eye movements present (%)	92.1 ± 3.1	75.3 ± 3.1†	53.7 ± 3.6*
Electromyographic activity			
Nuchal	1.43 ± 0.17	1.77 ± 0.10	1.25 ± 0.09
Biceps	1.14 ± 0.15	1.93 ± 0.10*	0.87 ± 0.08
Triceps	1.80 ± 0.17	2.65 ± 0.12*	1.42 ± 0.10‡
Tracheal pressure (%)	84.2 ± 4.2	85.3 ± 2.6	70.0 ± 3.3‡
Blood pressure (mm Hg)	41.0 ± 0.9	41.7 ± 0.6	41.4 ± 0.6
Fetal heart rate (bpm)	142.4 ± 1.6	145.8 ± 0.9	149.9 ± 1.2*
PaO <sub>2</sub> (mm Hg)	20.5 ± 0.3	20.1 ± 0.3	19.2 ± 0.2†

Electrocortical activity is represented as the percentage of time spent in each state. Eye movements and tracheal pressure are represented as the percentage of time during which rapid eye movements and fetal breathing were present. Electromyographic activity is represented as the mean number of electromyographic bursts occurring per 0.5-minute epoch. All values are represented as mean ± SEM for each consecutive 0.5-minute epoch analyzed (n = 19).

\*p < 0.001.

†p < 0.01.

‡p < 0.05.

cortical activity without eye movements or true low-voltage electrocortical activity with eye movements. The fetus had to undergo a complete electrocortical state cycle (that is, low-voltage, high-voltage, low-voltage) before another stimulation experiment was performed. In low-voltage electrocortical activity, 23 experiments were performed at a stimulation rate of 0.01 pps and 19 at 0.05 pps. In high-voltage electrocortical activity, 22 experiments were performed at a stimulation rate of 0.01 pps and 18 at 0.05 pps.

Data were analyzed in continuous 0.5-minute epochs. Electrocortical activity was classified as either low-voltage fast, high-voltage slow, or transitional. Eye movements and tracheal pressure were coded as either present or absent. Individual electromyographic bursts were counted for nuchal, biceps, and triceps muscle activity. Negative tracheal pressure deflections were used to identify the presence or absence of breathing activity. Mean arterial blood pressure and mean fetal heart rate were recorded for each 0.5-minute epoch. Intravascular PaO<sub>2</sub> was available from two sheep after 130 days and was measured in continuous 0.5-minute epochs. The data are represented as the mean ± SEM. Level of significance was determined by means of a *t* test for independent means.

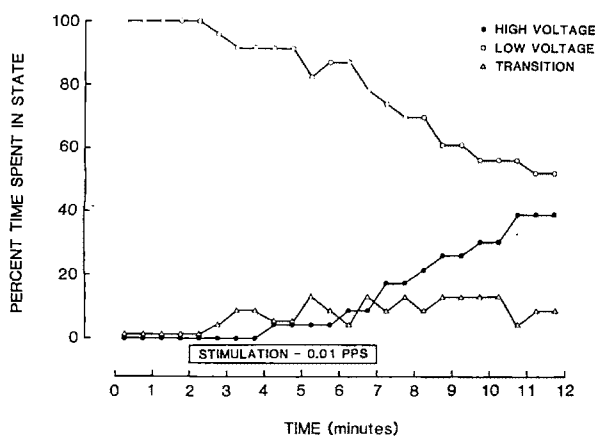
## Results

**Stimulation experiments in low-voltage electrocortical activity with eye movements at stimulation rates of 0.01 and 0.05 pps (Tables I and II).** Brachial nerve stimulation in low-voltage electrocortical activity with eye movements at 0.01 pps resulted in a 10% decrease in the mean percentage of time spent in low-voltage activity during the stimulation period and in a

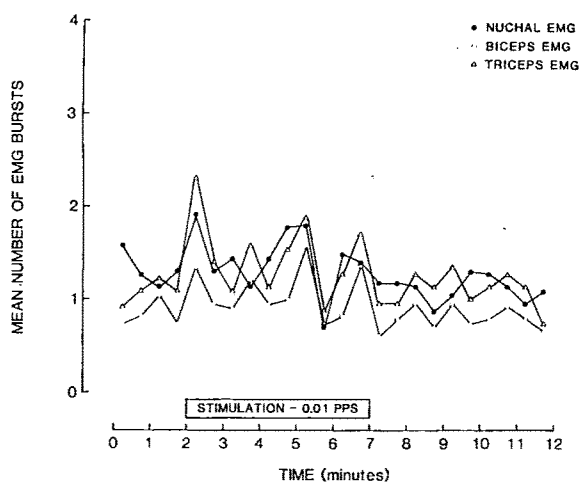
40% reduction in low-voltage activity during the recovery period (p < 0.001) (Fig. 1). There was a concomitant decrease in eye movement activity from 95.6% ± 2.1% in the control period to 79.1% ± 2.6% and 50.9% ± 3.3% in the stimulation and recovery periods, respectively (p < 0.001); that is, stimulation was associated with a state change. There was a slight but significant (p < 0.05) increase in biceps and triceps electromyographic activity (Fig. 2) during the stimulation period, which returned to control values during the recovery period. The incidence of fetal breathing decreased from 80.4% ± 4.2% in the control period to 70.0% ± 3.0% and 57.0% ± 3.2% in the stimulation and recovery periods, respectively (p < 0.001). These changes were in keeping with the alterations observed in fetal state. The fetal blood pressure remained constant while the fetal heart rate rose from 144.7 ± 1.5 bpm in the control period to 148.2 ± 0.9 bpm in the stimulation period to 152.7 ± 1.1 bpm in the recovery period (p < 0.001) (Fig. 3). There was no change in the intravascular PaO<sub>2</sub>.

Brachial nerve stimulation in low-voltage electrocortical activity with eye movements at 0.05 pps resulted in a 5% decrease in the percentage of time spent in low-voltage electrocortical activity during the stimulation period and a 44% decrease in the recovery period (p < 0.001) (Fig. 4). There was a concomitant decrease in eye movement activity from 92.1% ± 3.1% in the control period to 75.3% ± 3.1% and 53.7% ± 3.6% in the stimulation and recovery periods, respectively (p < 0.001); that is, stimulation led to a state change similar to that observed with the lower stimulus rate. There was an increase in nuchal, biceps, and triceps electromyographic activity that was much more pro-





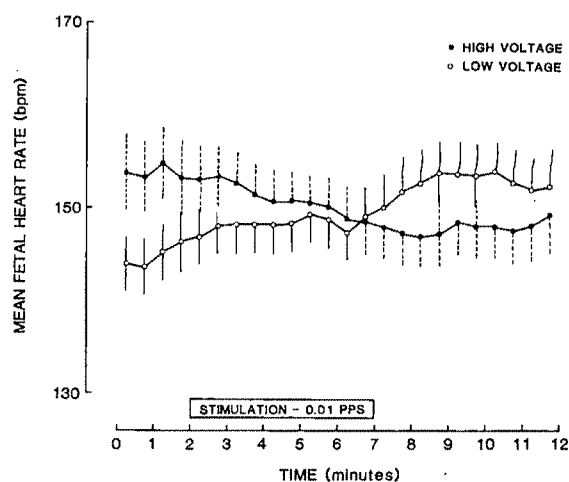
**Fig. 1.** The percentage of time spent in each state by fetuses during stimulation experiments at 0.01 pps beginning in low-voltage electrocortical activity with eye movements ( $n = 23$ ). The slope, or rate of change of low-voltage electrocortical activity during the stimulation period, was  $-3.7\%/min$  ( $r = -0.92$ ).



**Fig. 2.** The mean number of electromyographic (EMG) bursts per epoch demonstrated by fetuses during stimulation experiments at 0.01 pps beginning in low-voltage electrocortical activity with eye movements ( $n = 23$ ).

nounced than at the weaker stimulation rate (Fig. 5). These again returned to control values during the recovery period. The incidence of fetal breathing decreased from  $84.2\% \pm 4.2\%$  in the control period and  $85.3\% \pm 2.5\%$  during the stimulation period to  $70.0\% \pm 3.3\%$  ( $p < 0.05$ ) during the recovery period. The fetal blood pressure remained constant while the fetal heart rate increased from  $142.4 \pm 1.6$  to  $145.8 \pm 0.9$  to  $149.9 \pm 1.2$  bpm from control through stimulation to recovery, respectively ( $p < 0.001$ ) (Fig. 6). The  $PaO_2$  decreased from  $20.5 \pm 0.3$  mm Hg in the control period to  $19.2 \pm 0.2$  mm Hg during the recovery period ( $p < 0.01$ ).

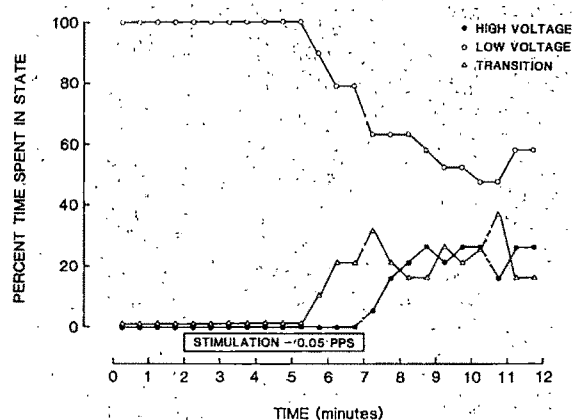
#### Stimulation experiments in high-voltage electro-



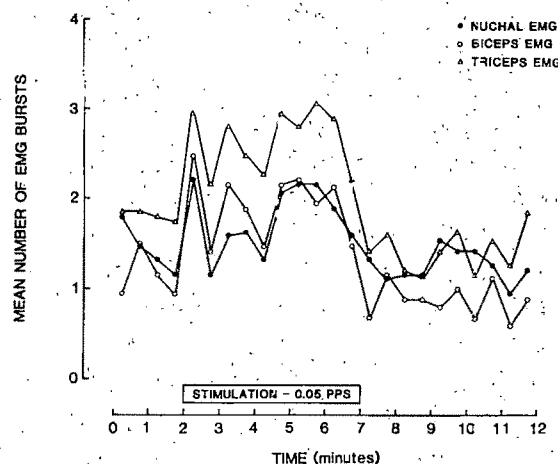
**Fig. 3.** Mean fetal heart rate (beats per minute) recorded from fetuses during stimulation experiments at 0.01 pps beginning in low-voltage electrocortical activity with eye movements ( $n = 23$ ) and in high-voltage electrocortical activity without eye movements ( $n = 22$ ).

**cortical activity without eye movements at stimulation rates of 0.01 and 0.05 pps (Tables III and IV).** Brachial nerve stimulation with 0.01 pps when the fetus was in high-voltage electrocortical activity with no rapid eye movements resulted in a 26% decrease in the percentage of time spent in high-voltage electrocortical activity during the stimulation period and a 45% decrease in the recovery period ( $p < 0.001$ ) (Fig. 7). There was a corresponding increase in eye movements from 0% in the control period to  $2.7\% \pm 1.1\%$  in the stimulation period to  $13.6\% \pm 2.3\%$  during the recovery period ( $p < 0.001$ ). The incidence of fetal breathing remained constant during the control and stimulation periods but increased significantly during the recovery period ( $p < 0.05$ ). There was no increase in nuchal, biceps, or triceps electromyographic activity (Fig. 8), nor was there a change in fetal blood pressure or intravascular  $PaO_2$  during the stimulation or the recovery period as compared with the control, while fetal heart rate (Fig. 3) demonstrated a slight decrease during the stimulation and recovery periods, respectively ( $p < 0.01$ ).

When the stimulus rate was increased to 0.05 pps in high-voltage electrocortical activity with no rapid eye movements, there was a dramatic decrease of 47% ( $p < 0.001$ ) in the incidence of high-voltage electrocortical activity during the stimulation period, which remained constant during the recovery period (Fig. 9). There was also a dramatic increase in the incidence of transitional electrocortical activity with a 10% incidence of rapid eye movements. The nuchal, biceps, and triceps electromyographic activity also increased significantly above control during the stimulation period ( $p < 0.001$ ) (Fig. 10). The incidence of fetal breathing



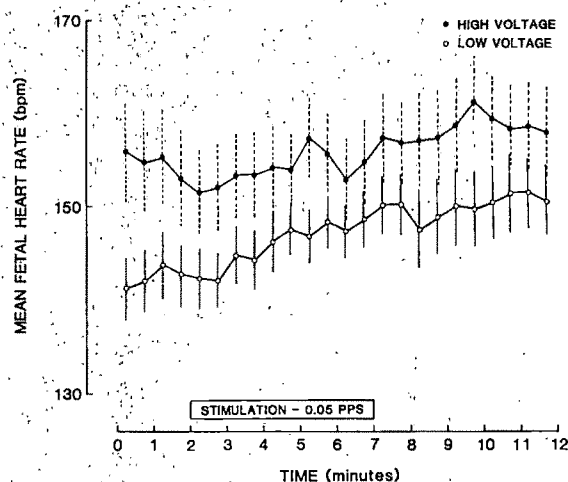
**Fig. 4.** The percentage of time spent in each state by fetuses during stimulation experiments at 0.05 pps beginning in low-voltage electrocortical activity with eye movements ( $n = 19$ ). The slope, or rate of change of low-voltage electrocortical activity during the stimulation period, was  $-4.0\%/min$  ( $r = -0.77$ ).



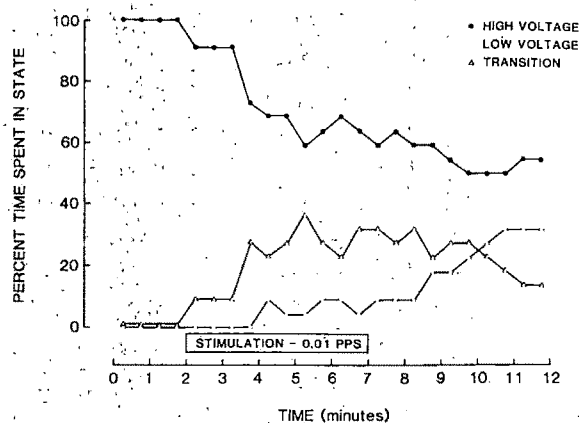
**Fig. 5.** The mean number of electromyographic (EMG) bursts per epoch demonstrated by fetuses during stimulation experiments at 0.05 pps beginning in low-voltage electrocortical activity with eye movements ( $n = 19$ ).

increased slightly during the stimulation period ( $p < 0.05$ ) but was not significantly different from control during the recovery period. However, the fetal blood pressure remained constant while the fetal heart rate changed only during the recovery period ( $p < 0.05$ ) (Fig. 6). The  $PaO_2$  decreased during the recovery period from  $20.1 \pm 0.1$  mm Hg to  $18.9 \pm 0.3$  mm Hg ( $p < 0.05$ ).

**Comparison of responsiveness to stimulation in high- and low-voltage electrocortical activity.** Examination of electrocortical activity during brachial nerve stimulation at a stimulation rate of 0.01 pps demonstrated that when the stimulation was initiated in high-voltage electrocortical activity without eye movements the fetus remained in high-voltage electrocortical ac-



**Fig. 6.** Mean fetal heart rate (beats per minute) recorded from fetuses during stimulation experiments at 0.05 pps beginning in low-voltage electrocortical activity with eye movements ( $n = 19$ ) and in high-voltage electrocortical activity without eye movements ( $n = 18$ ).



**Fig. 7.** The percentage of time spent in each state by fetuses during stimulation experiments at 0.01 pps beginning in high-voltage electrocortical activity without eye movements ( $n = 22$ ). The slope, or rate of change of high-voltage electrocortical activity during the stimulation period, was  $-7.7\%/min$  ( $r = -0.90$ ).

tivity 73.6% of the time, as compared with stimulations that were initiated in low-voltage electrocortical activity with eye movements, where the fetus remained in low-voltage electrocortical activity 89.6% of the time ( $p < 0.005$ ). This change was predominantly in favor of transitional electrocortical activity ( $p < 0.001$ ). The rate of change of state during the stimulation period in high-voltage electrocortical activity without eye movements was  $-7.7\%/min$ , while in low-voltage electrocortical activity with eye movements it was  $-3.7\%/min$  ( $p < 0.01$ ). During the recovery phase the fetus remained in the initial state more often when the stimulation began in low-voltage electrocortical activity with eye movements

**Table III.** Stimulation experiments: high-voltage electrocortical activity, stimulation rate = 0.01 pps

	Control	Stimulation	Recovery
Electrocortical activity (%)			
High	100.0	73.6 ± 3.9*	55.5 ± 1.5*
Low	0.0	4.1 ± 1.3	20.9 ± 3.0*
Transitional	0.0	22.3 ± 3.1*	23.6 ± 2.1*
Eye movements present (%)	0.0	2.7 ± 1.1	13.6 ± 2.3*
Electromyographic activity			
Nuchal	1.36 ± 0.14	1.13 ± 0.07	1.48 ± 0.09
Biceps	0.88 ± 0.11	0.92 ± 0.06	0.91 ± 0.08
Triceps	1.43 ± 0.13	1.31 ± 0.08	1.41 ± 0.09
Tracheal pressure (%)	9.1 ± 3.1	8.2 ± 1.9	21.4 ± 2.8†
Blood pressure (mm Hg)	38.5 ± 0.7	38.7 ± 0.4	38.8 ± 0.4
Fetal heart rate (bpm)	153.7 ± 1.8	150.9 ± 1.0	147.8 ± 1.1‡
PaO <sub>2</sub> (mm Hg)	20.3 ± 0.2	20.5 ± 0.1	20.6 ± 0.1

Electrocortical activity is represented as the percentage of time spent in each state. Eye movements and tracheal pressure are represented as the percentage of time during which rapid eye movements and fetal breathing were present. Electromyographic activity is represented as the mean number of electromyographic bursts occurring per 0.5-minute epoch. All values are represented as mean ± SEM for each consecutive 0.5-minute epoch analyzed (n = 22).

\*p < 0.001.

†p < 0.05.

‡p < 0.01.

**Table IV.** Stimulation experiments: high-voltage electrocortical activity, stimulation rate = 0.05 pps

	Control	Stimulation	Recovery
Electrocortical activity (%)			
High	100.0	53.3 ± 4.6*	53.9 ± 2.6*
Low	0.0	15.0 ± 3.5*	10.6 ± 2.4*
Transitional	0.0	31.7 ± 3.1*	35.5 ± 2.6*
Eye movements present (%)	0.0	9.4 ± 2.2†	8.9 ± 2.1†
Electromyographic activity			
Nuchal	1.18 ± 0.13	2.07 ± 0.08*	1.02 ± 0.08
Biceps	0.63 ± 0.11	1.71 ± 0.08*	0.74 ± 0.07
Triceps	0.99 ± 0.14	2.42 ± 0.10*	1.21 ± 0.10
Tracheal pressure (%)	18.1 ± 4.6	28.9 ± 3.7	25.0 ± 3.2
Blood pressure (mm Hg)	39.9 ± 0.7	41.3 ± 0.5	41.1 ± 0.5
Fetal heart rate (bpm)	154.7 ± 2.6	153.8 ± 1.3	158.2 ± 1.5‡
PaO <sub>2</sub> (mm Hg)	20.1 ± 0.1	19.8 ± 0.2	18.9 ± 0.3‡

Electrocortical activity is represented as the percentage of time spent in each state. Eye movements and tracheal pressure are represented as the percentage of time during which rapid eye movements and fetal breathing were present. Electromyographic activity is represented as the mean number of electromyographic bursts occurring per 0.5-minute epoch. All values are represented as mean ± SEM for each consecutive 0.5-minute epoch analyzed (n = 18).

\*p < 0.001.

†p < 0.01.

‡p < 0.05.

(60.9%) as compared with that in high-voltage electrocortical activity without eye movements (55.4%) (p < 0.1). However, fetuses initially stimulated in high-voltage electrocortical activity without eye movements spent significantly more time in transitional electrocortical activity during the recovery period (p < 0.001).

When fetuses were stimulated at 0.05 pps, it was again noted that the electrocortical state changed significantly more often in fetuses during the stimulation period in high-voltage electrocortical activity without eye movements (53.3%) than during low-voltage electrocortical activity with eye movements (94.7%) (p < 0.001). This change was also in favor of transitional

electrocortical activity (p < 0.001). The rate of change of state was -10.0%/min in high-voltage electrocortical activity without eye movements and -4.0%/min in low-voltage electrocortical activity with eye movements during the stimulation period (p < 0.001).

During the recovery period there was no difference noted in the proportion of time spent in the initial electrocortical state between stimulations in high-voltage electrocortical activity without eye movements and those in low-voltage electrocortical activity with eye movements. However, in fetuses stimulated initially in high-voltage electrocortical activity without eye movements as compared with those stimulated in low-voltage

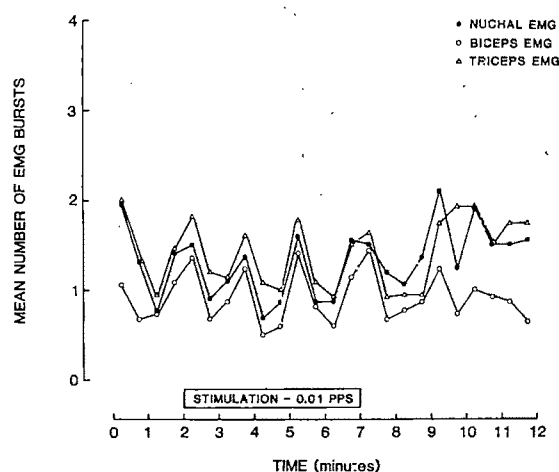


Fig. 8. The mean number of electromyographic (EMG) bursts per epoch demonstrated by fetuses during stimulation experiments at 0.01 pps beginning in high-voltage electrocortical activity without eye movements ( $n = 22$ ).

electrocortical activity with eye movements, again significantly more time was spent in transitional electrocortical activity during recovery.

Examination of electromyographic activity during stimulation in high-voltage electrocortical activity without eye movements and low-voltage electrocortical activity with eye movements demonstrated that at a stimulation rate of 0.05 pps the percentage change in nuchal electromyographic activity was 75.4 in high-voltage electrocortical activity and 23.8 in low-voltage electrocortical activity. There was an increase in biceps electromyographic activity of 171.4% in high-voltage electrocortical activity as compared with 69.3% in low-voltage electrocortical activity. Similarly, the percentage increase for triceps electromyographic activity was 144.0 in high-voltage electrocortical activity as compared with 47.2 in low-voltage electrocortical activity. This supports the hypothesis that muscle is more responsive to stimulation in high-voltage electrocortical activity.

It is of note that the fetal heart rate was significantly lower in the control periods of low-voltage electrocortical activity with eye movements than with high-voltage electrocortical activity without eye movements (144.7 versus 153.7 bpm) at both stimulation rates ( $p < 0.01$ ).

#### Comment

These experiments have demonstrated that during low-voltage electrocortical activity with rapid eye movements, the application of somatosensory stimuli to the brachial nerve produced only a minimal increase in biceps and triceps electromyographic activity, which was enhanced when an increased stimulation rate was applied. When the same stimuli were applied while the

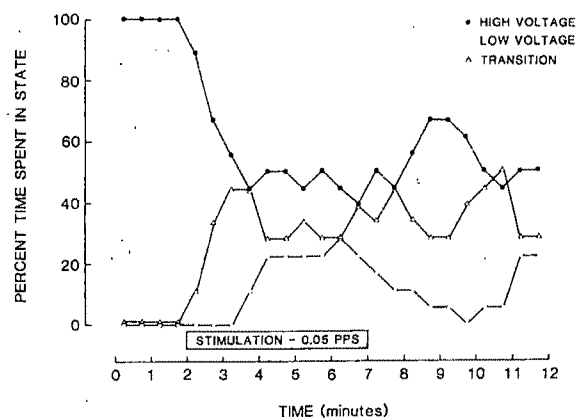


Fig. 9. The percentage of time spent in each state by fetuses during stimulation experiments at 0.05 pps beginning in high-voltage electrocortical activity without eye movements ( $n = 18$ ). The slope, or rate of change of high-voltage electrocortical activity during the stimulation period, was  $-10.0\%/min$  ( $r = -0.84$ ).

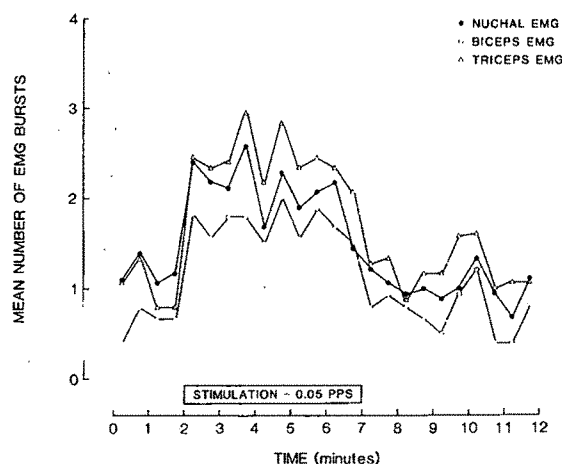


Fig. 10. The mean number of electromyographic (EMG) bursts per epoch demonstrated by fetuses during stimulation experiments at 0.05 pps beginning in high-voltage electrocortical activity without eye movements ( $n = 18$ ).

fetus was in high-voltage electrocortical activity without eye movements, a marked enhancement of biceps, triceps, and nuchal muscle activity was observed at the higher stimulation rate. Natale et al.<sup>14</sup> demonstrated increased fetal forelimb movements during high-voltage electrocortical activity and transitional states. In this experiment, because we observed a state change, the slight increases in biceps and triceps electromyographic activity observed with low stimulation rates during low-voltage activity may have simply been a reflection of the state changes observed. Moreover, because the electromyographic and state changes were not as profound when the higher stimulation rate was applied in low-voltage electrocortical activity with eye movements as compared with high-voltage electrocortical activity



without eye movements, it is possible to conclude that the fetus is less arousable when in a state of low-voltage electrocortical activity with eye movements. This is consistent with findings by other investigators.<sup>12, 15</sup>

It was of note, however, that the blood pressure as well as the intravascular  $\text{PaO}_2$  at both stimulus rates in both behavioral states remained constant during the control and stimulation periods. Although a change in  $\text{PaO}_2$  was demonstrated in the recovery period of stimulations at 0.05 pps in high- and low-voltage activity, we suspect that these changes are unrelated to the stimulation experiments and may reflect uterine contractions.<sup>16</sup> The fetal heart rate demonstrated a difference according to behavioral state and stimulus strength. In high-voltage electrocortical activity without eye movements there was no change in fetal heart rate during the stimulation, whereas in low-voltage electrocortical activity with eye movements there was an increase in fetal heart rate during the stimulation period, which became enhanced during the recovery period.

From the data presented, the control fetal heart rate was approximately 10 bpm less when the fetus was in low-voltage electrocortical activity with eye movements as compared with high-voltage electrocortical activity without eye movements, suggesting that the increase in fetal heart rate that was observed during the stimulation in low-voltage electrocortical activity with eye movements may have been secondary to the observed alteration in behavioral state. The increase in fetal heart rate seen during recovery periods at both stimulation rates, with the exception of the stimulation rate of 0.01 pps in high-voltage electrocortical activity without eye movements, may be a result of the magnitude of the stimulus applied and, therefore, may reflect catecholamine release in response to induced fetal pain. In clinical obstetrics, an increase in heart rate after stimulation of the fetus in utero has been used as an index of fetal health. From our experiments, it is difficult to find support for this hypothesis, since stimulations during high-voltage electrocortical activity without eye movements did not produce significant changes in fetal heart rate.

It is concluded that the behavioral state of the fetus before stimulation is an important determinant of observed responses in measurements of fetal breathing, fetal body movements, and fetal heart rate under conditions of normoxia. Similar studies are required under conditions of fetal hypoxemia and acidemia before con-

clusions can be drawn of how behavioral states affect parameters of clinical fetal well-being.

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# Effects of reduced uterine blood flow on accelerations and decelerations in heart rate of fetal sheep

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Experiments were conducted in unanesthetized fetal sheep during the last third of gestation to examine the effects of prolonged reversible reductions in uterine blood flow on mean fetal heart rate, accelerations and decelerations in fetal heart rate, and fetal arterial pressure. With use of a Teflon vascular clamp placed around the maternal common internal iliac artery, uterine blood flow was reduced, leading to a reduction in fetal arterial oxygen saturation from 60% to 30% for 2 hours. This was associated with an initial transient fetal bradycardia and hypertension followed by tachycardia. Mean fetal heart rate remained significantly elevated for 2 hours following the release of the vascular clamp. There was no change in the number, amplitude, or duration of accelerations, but there was a significant increase in both the number and amplitude of decelerations during the period of reduced uterine blood flow. (AM J OBSTET GYNECOL 1986;154:329-35.)

**Key words:** Heart rate, fetal sheep, uterine blood flow, hypoxemia

The presence of accelerations in antepartum fetal heart rate is indicative of well-being in the human fetus.<sup>1,2</sup> The absence of these accelerations is associated with fetal acidosis and a significant increase in perinatal morbidity and mortality.<sup>1,2</sup> Little is known, however, about the normal progression of changes in heart rate variability with prolonged hypoxemia that may occur as a result of reduced uterine blood flow.

We have previously reported that accelerations and decelerations in fetal heart rate are normally present in the sheep fetus during the last third of gestation.<sup>3</sup> With use of a computer analysis similar to that described for the human fetal heart rate,<sup>4</sup> we have now quantitatively assessed the characteristics of accelerations and decelerations in the fetal heart rate of sheep during prolonged reversible reductions in uterine blood flow. Previous studies of fetal heart rate variability in sheep during hypoxemia have been conducted by reducing oxygen delivery to the fetus as a consequence of maternal hypoxemia.<sup>5,6</sup> By mechanically reducing uterine blood flow in chronically catheterized sheep, we have been able to produce prolonged fetal hypoxemia without the maternal metabolic and endocrine changes associated with having the ewe inspire a

reduced oxygen gas mixture. These experiments were therefore carried out to examine the effects of mild fetal hypoxemia on fetal heart rate accelerations and decelerations, as well as the mean heart rate and blood pressure, in unanesthetized fetal sheep during the last third of gestation.

## Material and methods

**Animal preparation.** Surgery was performed under aseptic conditions in seven pregnant ewes of known mating dates between 105 and 116 days gestation (mean: 110 days). Under halothane anesthesia, a midline ventral incision was made in the ewe's abdomen and a Teflon vascular clamp was placed around the maternal common internal iliac artery. An incision was then made in the uterus, and polyvinyl catheters (Dural Plastics, Australia, SV65) were placed in either the fetal carotid artery and jugular vein ( $n = 4$ ) or descending aorta and inferior vena cava ( $n = 3$ ). Fine stainless steel electrodes (Cooner Sales Company, U.S.A., No. AS632) were sutured subcutaneously over the fetal sternum to record the fetal electrocardiogram. In addition, pairs of electrodes were placed over the parietal dura and in the nuchal and limb muscles, and a polyvinyl catheter was inserted into the fetal trachea for observations that are the subject of a separate study. In all animals, polyvinyl catheters (Dural Plastics, SV116) were placed in the amniotic sac as well as in the maternal carotid artery and jugular vein. At the end of the surgical procedure the control cable of the vascular clamp and all fetal catheters and wires were exteriorized through the ewe's flank, and antibiotics (benzylpenicillin, 400,000 U and streptomycin, 500 mg) were injected into the amniotic sac.

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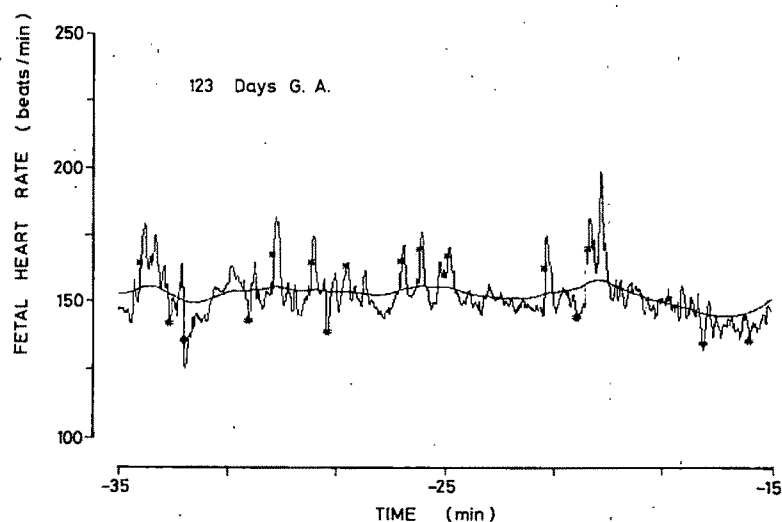


Fig. 1. Typical example of fetal heart rate over 20 minutes in a sheep fetus at 123 days' gestation. The baseline derived from the digital filter is superimposed, and the symbols (\*, #) indicate, respectively, all fetal heart rate accelerations and decelerations that are  $\geq 10$  bpm away from the baseline lasting for 5 seconds or longer.

Table I. Fetal and maternal percent oxygen saturation and arterial blood gases before ( $-15$  min), during ( $+15$ ,  $+60$ ,  $+120$  min), and after ( $p30$  min) the 2-hour period of reduced uterine blood flow

	$-15$ min	$+15$ min	$+60$ min	$+120$ min	$p30$ min
Fetal					
SaO <sub>2</sub> (%)	61.1 $\pm$ 2.1	26.9 $\pm$ 1.7*	30.9 $\pm$ 1.7*	30.8 $\pm$ 2.2*	53.5 $\pm$ 2.7†
PO <sub>2</sub> (mm Hg)	22.1 $\pm$ 0.6	14.5 $\pm$ 0.9*	15.8 $\pm$ 0.9*	16.4 $\pm$ 0.9*	20.6 $\pm$ 0.8‡
PCO <sub>2</sub> (mm Hg)	45.5 $\pm$ 1.5	50.5 $\pm$ 2.0‡	49.3 $\pm$ 1.4†	47.0 $\pm$ 1.4	43.8 $\pm$ 1.4
pH	7.34 $\pm$ 0.1	7.27 $\pm$ 0.01*	7.25 $\pm$ 0.01†	7.25 $\pm$ 0.01†	7.31 $\pm$ 0.01‡
Maternal					
SaO <sub>2</sub> (%)	93.4 $\pm$ 0.7	94.3 $\pm$ 0.4	94.8 $\pm$ 0.7	94.6 $\pm$ 0.5	94.1 $\pm$ 0.6
PO <sub>2</sub> (mm Hg)	98.9 $\pm$ 4.4	104.1 $\pm$ 2.8	101.7 $\pm$ 3.8	103.1 $\pm$ 1.7	101.4 $\pm$ 3.1
PCO <sub>2</sub> (mm Hg)	28.3 $\pm$ 1.0	29.5 $\pm$ 1.2	29.9 $\pm$ 1.8	29.8 $\pm$ 1.2	28.5 $\pm$ 1.0
pH	7.5 $\pm$ 0.01	7.5 $\pm$ 0.01	7.49 $\pm$ 0.01	7.49 $\pm$ 0.01	7.49 $\pm$ 0.01

Results are mean values  $\pm$  SEM ( $n = 14$ ).

\* $p < 0.001$ .

† $p < 0.01$ .

‡ $p < 0.05$  (paired  $t$  test for comparison to  $t = -15$ ).

The ewes were housed in individual cages with free access to food and water and were allowed 5 days to recover from surgery before any experiments were performed. Six fetuses survived until term (140 to 145 days' gestation), and one ewe (with a healthy fetus) was electively killed at 135 days' gestation. The mean fetal weight at the end of the experiments was  $3.7 \pm 0.5$  kg.

**Experimental protocol.** All experiments began at 10 AM and consisted of an initial 2-hour data collection period, after which uterine blood flow was reduced to produce a fetal arterial oxygen saturation (SaO<sub>2</sub>) of approximately 30%. This degree of reduction in SaO<sub>2</sub> was maintained for 2 hours, and then the vascular clamp was released and recordings were continued for an additional 2 hours.

Fetal and maternal arterial blood samples were ob-

tained 15 minutes before and 15, 60, and 120 minutes after the onset of the reduction in uterine blood flow and 30 minutes after the release of the vascular clamp. Control experiments were conducted in the same fetuses at the same time of day and at similar gestational ages. The same data collection procedure was followed although no manipulation of the vascular clamp was performed. Each animal had an average of four occlusion experiments performed (range: two to eight), and at least 48 hours was allowed between successive experiments performed in the same fetus.

**Data analysis.** Fetal heart rate was derived from the fetal electrocardiogram with use of a microprocessor system that measured successive R-R intervals to an accuracy of  $\pm 1$  msec. An error rejection algorithm excluded any intervals that were  $< 80\%$  or  $> 120\%$  of the

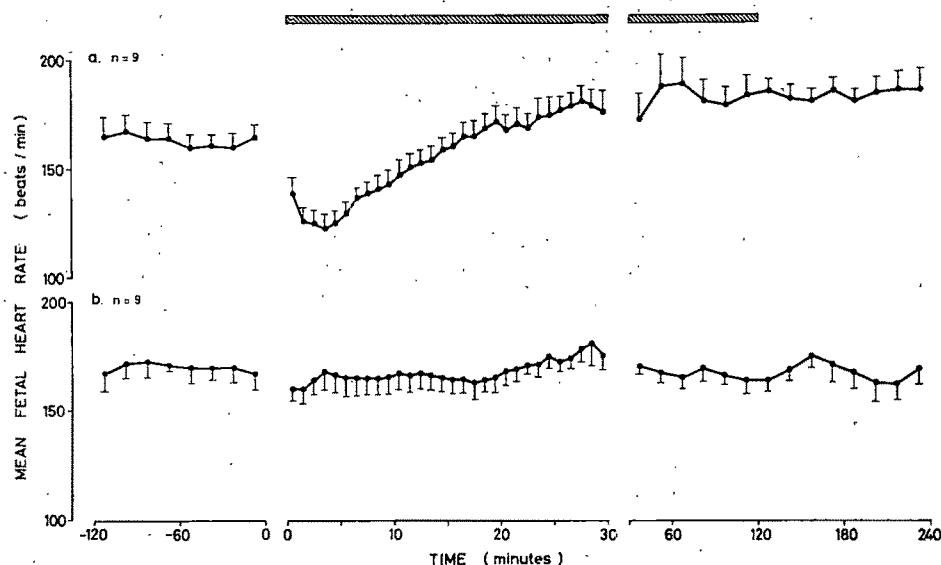


Fig. 2. Mean fetal heart rate for nine experiments in six fetuses when uterine blood flow was reduced (a) and nine experiments in the same fetuses when uterine blood flow was not altered (b). Results are means  $\pm$  SEM, and the hatched bar at the top indicates the time during which uterine blood flow was reduced.

previous valid interval. The valid R-R intervals were averaged over 1-second epochs and then transferred to a large time-shared computer system (VAX 11/780, DEC Corporation) for storage and later analysis. In addition, an analogue output of fetal heart rate from the microprocessor was displayed on a Grass polygraph. Accelerations and decelerations were defined as transient increases and decreases in fetal heart rate from the baseline of  $\geq 10$  bpm lasting for 5 seconds or longer. The baseline fetal heart rate was determined with use of a recursive low-pass digital filter similar to that described by Dawes et al.<sup>4</sup> over successive 60-minute episodes. Fig. 1 demonstrates a typical example of the sheep fetal heart rate at 123 days' gestation with the baseline superimposed. Data from the first 15 minutes of reduced uterine blood flow were excluded from the analysis of accelerations and decelerations because of the profound bradycardia observed at this time. The number of 1-second epochs that were excluded because of artifact were 1.6% and 2.2% of the total time for occlusion and control experiments, respectively.

Fetal arterial and amniotic pressures were measured with use of standard pressure transducers (Gould Statham Instruments, P23 ID) and purpose-built pressure amplifiers. Arterial pressure, with amniotic pressure electronically subtracted from it, was continuously recorded on the polygraph. Fetal arterial systolic and diastolic pressures were measured from polygraph recordings at 1-minute intervals for the first 30 minutes of reduced uterine blood flow and at 5-minute intervals for the remainder of the study period.

Arterial  $PO_2$ ,  $PCO_2$ , and pH were measured with use

of either a Corning 165/2 or Radiometer ABL30 blood gas analyzer and then corrected for a fetal temperature of 39° C. Arterial  $SAO_2$  was measured with use of a Radiometer OSM2 Oximeter. All results are presented as means  $\pm$  SEM, and statistical significance was determined by means of a paired *t* test. When multiple experiments were performed in one animal, the means for the results were found for each fetus before performing the statistical analysis.

## Results

**Blood gases and  $SAO_2$ .** We performed 14 experiments in the seven fetuses between 111 and 142 days' gestation (mean: 125 days) in which uterine blood flow was reduced. Fetal  $SAO_2$  fell from  $61.1\% \pm 2.1\%$  during the control period to  $29.3\% \pm 1.6\%$  during the 2 hours of reduced uterine blood flow, representing a decrease of 52% (Table I). Fetal arterial  $PO_2$  and pH also decreased during the period of reduced uterine blood flow and remained significantly depressed at 30 minutes after the release of the vascular clamp. Although fetal arterial  $PCO_2$  increased slightly from  $45.5 \pm 1.5$  to  $48.7 \pm 1.4$  mm Hg, this was statistically significant only during the first 60 minutes of reduced uterine blood flow.

There was no significant change in maternal arterial blood gases or  $SAO_2$  during the period of reduced uterine blood flow (Table I). During eight control experiments in six fetuses the fetal arterial  $SAO_2$ ,  $PO_2$ ,  $PCO_2$ , and pH were  $57.5\% \pm 2.6\%$ ,  $21.6 \pm 0.7$  mm Hg,  $44.5 \pm 2.2$  mm Hg, and  $7.35 \pm 0.02$ , respectively.

**Mean fetal heart rate and arterial pressure.** Mean fetal heart rate fell from  $162.8 \pm 6.3$  bpm during the



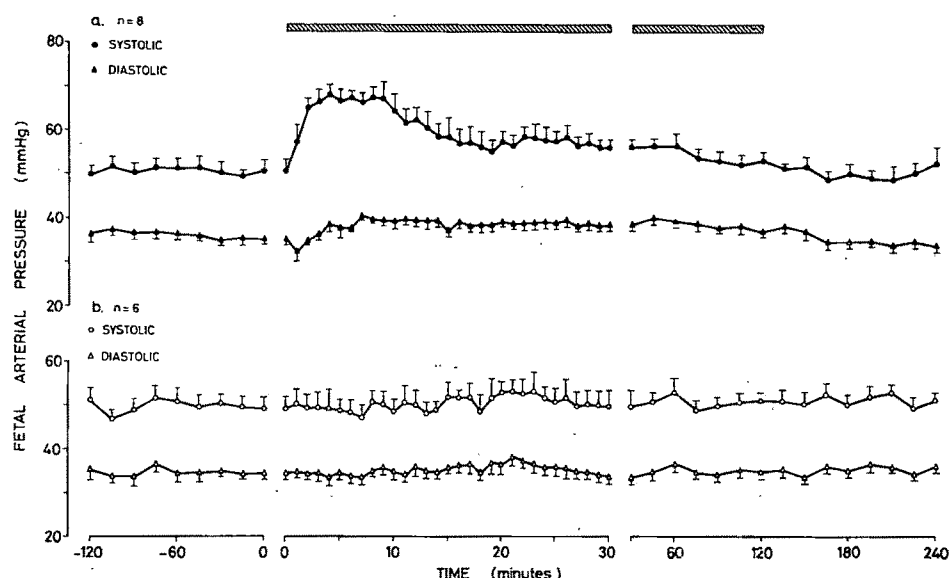


Fig. 3. Fetal arterial systolic (—●—) and diastolic (—▲—) pressure for eight experiments in five fetal sheep when uterine blood flow was reduced (a) and six control experiments in five fetal sheep when uterine blood flow was not altered (b). The hatched bar at the top indicates the time during which uterine blood flow was reduced.

Table II. Mean values ( $\pm$  SEM) for the number, amplitude, and duration of accelerations and decelerations in heart rate for nine experiments in six fetal sheep measured before ( $-120 \rightarrow 0$  min), during ( $0 \rightarrow 120$  min), and after ( $120 \rightarrow 240$  min) a 2-hour period of reduced uterine blood flow

	$-120 \rightarrow 0$ min	$0 \rightarrow 120$ min	$120 \rightarrow 240$ min
Accelerations			
Number per hour	$36.4 \pm 4.1$	$36.1 \pm 5.4$	$43.3 \pm 3.3$
Amplitude (bpm)	$22.9 \pm 1.3$	$19.2 \pm 1.2$	$23.4 \pm 1.6$
Duration (sec)	$14.7 \pm 1.0$	$14.7 \pm 1.2$	$14.6 \pm 1.0$
Decelerations			
Number per hour	$22.9 \pm 4.9$	$38.7 \pm 5.9^*$	$40.0 \pm 5.9^*$
Amplitude (bpm)	$15.5 \pm 0.8$	$18.4 \pm 1.2^\dagger$	$17.2 \pm 0.9$
Duration (sec)	$18.8 \pm 2.4$	$14.8 \pm 1.1$	$16.4 \pm 1.1$

\* $p < 0.02$ .

$^\dagger p < 0.05$  (paired  $t$  test for comparison to values during the 120 minutes before the period of reduced uterine blood flow).

control period to  $138.3 \pm 6.7$  bpm within the first 60 seconds of reduced uterine blood flow in nine experiments in six fetuses between 117 and 142 days' gestation (Fig. 2). The lowest mean fetal heart rate of  $122.1 \pm 5.9$  bpm occurred at 4 minutes, representing a mean decrease of 33% (range: 25% to 42%). An initial bradycardia followed by a gradual increase in mean fetal heart rate was seen in all experiments. Fifteen minutes after the onset of the reduction in uterine blood flow, mean fetal heart rate had returned to control levels; by 120 minutes, it had risen to  $183.3 \pm 9.0$  bpm, which was significantly greater than during the control period ( $p < 0.05$ ). Mean fetal heart rate remained significantly elevated ( $187.0 \pm 5.0$  bpm) during the recovery period after the vascular clamp was released ( $p < 0.05$ ), representing an increase of 16%

(range: 3% to 35%) over control levels. Mean fetal heart rate was  $170.4 \pm 6.1$  bpm during nine control experiments in the same six fetuses between 116 and 141 days' gestation, and there was no significant change over the 6 hours of observation (Fig. 2).

Fetal arterial pressure was recorded during eight experiments in five fetuses between 111 and 131 days' gestation when uterine blood flow was reduced. Mean systolic pressure increased from  $50.7 \pm 1.8$  mm Hg during the control period to a maximum of  $68.4 \pm 1.7$  mm Hg at 4 minutes after the onset of the reduction in uterine blood flow ( $p < 0.001$ ). This was an increase of 38% (range: 26% to 52%) over control levels (Fig. 3). Systolic pressure remained significantly elevated for 10 minutes and then gradually decreased to  $56.6 \pm 1.0$  mm Hg between 15 and 60 minutes after the onset of

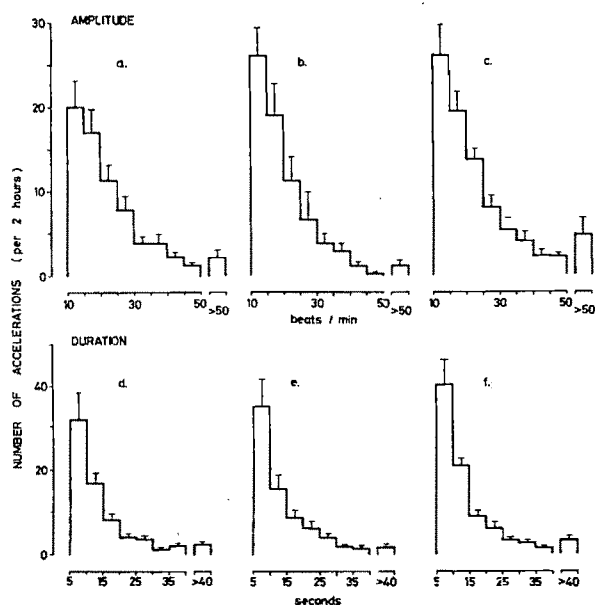


Fig. 4. Frequency histograms of the amplitude (a, b, c) and duration (d, e, f) of accelerations for the 2 hours before (a, d), 2 hours during (b, e), and the 2 hours after (c, f) the reduction in uterine blood flow.

reduced uterine blood flow, which was not significantly different from control values. There was no significant elevation in fetal systolic pressure during the latter 60 minutes of the period of reduced uterine blood flow or in the recovery period.

Diastolic pressure increased gradually from  $35.9 \pm 1.1$  mm Hg during the control period to  $40.2 \pm 1.2$  mm Hg at 7 minutes after uterine blood flow was reduced ( $p < 0.002$ ), as shown in Fig. 3, representing an increase of 11% (range: 7% to 18%) over control levels. In contrast to systolic pressure, diastolic pressure remained significantly elevated for the first 60 minutes of the period of reduced uterine blood flow ( $p < 0.05$ ). Arterial blood pressure was recorded throughout six control experiments in five fetuses between 110 and 125 days' gestation. There was no significant change in either systolic or diastolic pressure over the 6-hour period, as is shown in Fig. 3.

**Accelerations and decelerations.** The number of accelerations per hour ( $36.4 \pm 4.1$  during the control period) did not change either when uterine blood flow was reduced or during the recovery period (Table II). Similarly, the mean amplitude of accelerations ( $22.9 \pm 1.3$  bpm during the control period) did not change when uterine blood flow was reduced (Table II). There was, however, a significant decrease ( $p < 0.05$ ) in the proportion of accelerations that were 45 to 50 bpm in amplitude, from 2.4% in the control period to 0.3% during the period of reduced uterine blood flow. There was no other significant change in the frequency dis-

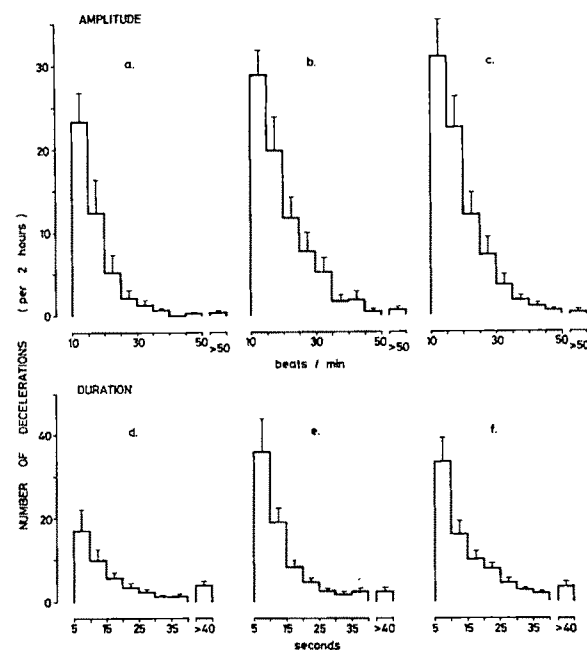


Fig. 5. Frequency histograms of the amplitude (a, b, c) and duration (d, e, f) of decelerations for the 2 hours before (a, d), 2 hours during (b, e), and 2 hours after (c, f) the reduction in uterine blood flow.

tribution of acceleration amplitudes both during and after the period of reduced uterine blood flow when compared to the control period (Fig. 4). The mean duration of accelerations ( $14.7 \pm 1.0$  seconds during the control period) did not change significantly when uterine blood was reduced (Table II), and there was no significant change in the frequency distribution of acceleration durations (Fig. 4).

The incidence of decelerations ( $22.9 \pm 4.9$  per hour during the control period) increased significantly to  $38.7 \pm 5.9$  per hour ( $p < 0.05$ ) when uterine blood flow was reduced and remained significantly elevated ( $p < 0.05$ ) during the recovery period (Table II). The mean amplitude of decelerations increased from  $15.5 \pm 0.8$  bpm to  $18.4 \pm 1.2$  bpm when uterine blood flow was reduced ( $p < 0.02$ ), and although it remained elevated at  $17.2 \pm 0.9$  bpm during the recovery period, this was not significantly different from control values. The only significant change in the frequency distribution of deceleration amplitudes was an increase in the proportion of decelerations that were 25 to 30 bpm, which changed from 4.8% to 9.4% when uterine blood flow was reduced and to 9.3% during the recovery period (Fig. 5).

The mean duration of decelerations ( $18.8 \pm 2.4$  seconds during the control period) was not significantly altered when uterine blood flow was reduced, although there was a small decrease (Table II). There was no

change in the frequency distribution of the duration of decelerations (Fig. 5).

### Comment

These experiments demonstrate that the major cardiovascular changes in the sheep fetus during prolonged reductions in uterine blood flow are an initial profound bradycardia and hypertension followed by tachycardia and normotension. In association with the prolonged tachycardia is an increase in both the number and amplitude of fetal heart rate decelerations. However, we found no change in the number, amplitude, or duration of fetal heart rate accelerations. Although we did not measure uterine blood flow in these experiments, previous studies with use of a technique similar to ours, but in combination with electromagnetic flowmeters placed around the middle uterine arteries, have demonstrated that blood flow to the pregnant uterus is reduced when the maternal common internal iliac artery is partially constricted.<sup>7</sup> Our aim was to use this method to produce a prolonged moderate reduction in fetal arterial oxygen levels.

An increase in fetal heart rate variability has previously been observed in fetal sheep during maternal hypoxemia<sup>5,6</sup> although the relative contribution of accelerations and decelerations to this increase has not been addressed. Dalton et al.<sup>5</sup> observed an 80% increase in both beat-to-beat variability and the 2-minute standard deviation of heart periods during 1 hour of maternal hypoxemia. Our results suggest that the observed increase in the 2-minute standard deviation during hypoxemia is largely a result of an increase in the decelerative components of heart rate variability. Parer et al.<sup>6</sup> also observed an increase in the oscillation frequency of heart rate variation and fetal heart rate amplitude variability during shorter periods of maternal hypoxemia. In contrast to those experiments, we did not observe any major changes in the frequency distribution of amplitudes of either accelerations or decelerations during prolonged periods of reduced uterine blood flow. This may be explained by the fact that we excluded the first 15 minutes of reduced blood flow data from our analysis whereas Parer et al.<sup>6</sup> observed a shift in fetal heart rate amplitude variability at 5 minutes of maternal hypoxemia.

In fetal sheep the accelerations in heart rate are closely associated temporally with skeletal muscle activity. The majority of accelerations occur with skeletal muscle activity as a consequence of central neuronal output apparently affecting both the cardioaccelerator fibers and skeletal muscle motoneurons simultaneously.<sup>3</sup> It is of interest therefore that the number of accelerations in heart rate is not altered during mild hypoxemia resulting from reduced uterine blood flow whereas the same degree of hypoxemia leads to a sig-

nificant reduction in skeletal muscle activity.<sup>8</sup> The explanation for this apparent difference in the cardiovascular and skeletal muscle responses to prolonged, mild hypoxemia is not clear. It is possible that hypoxia may have its effect on skeletal muscle activity at different sites centrally than those involved in cardiovascular control. In addition, a more severe degree of hypoxemia may be necessary to completely abolish all accelerations and decelerations in heart rate. It has previously been reported that during prolonged maternal hypoxemia, beat-to-beat variability in fetal heart rate does not decrease until severe acidosis occurs.<sup>5</sup> The effect of more severe hypoxia and acidosis on fetal heart rate accelerations and decelerations in sheep remains unknown, however.

The initial bradycardia that we observed in our experiments is similar to that which follows brief complete cessations of uterine blood flow<sup>9</sup> as well as during acute fetal hypoxemia resulting from induced maternal hypoxemia.<sup>10,11</sup> The decrease in fetal heart rate has been attributed to activation of the aortic chemoreceptors, since it can be abolished either by vagotomy<sup>10</sup> or pretreatment of the fetus with a cholinergic blocker, atropine.<sup>9</sup> The transient increase in fetal arterial pressure which we observed during the first 10 minutes of reduced uterine blood flow has also been described previously.<sup>9,11</sup> The increase in arterial pressure following brief complete cessations of uterine blood flow may be abolished by pretreatment with an  $\alpha$ -adrenergic blocking agent, phentolamine.<sup>9</sup> Similarly, the increase in arterial pressure in fetal hypoxemia secondary to maternal hypoxemia can be abolished<sup>12</sup> or significantly reduced<sup>13</sup> by pretreatment with phentolamine. During hypoxemia, there is a reduction in blood flow to the splanchnic, renal, and carcass vascular beds indicative of a systemic vasoconstriction that leads to the increase in arterial pressure.<sup>11</sup> Recently, Itskovitz and Rudolph<sup>14</sup> have confirmed that the fetal cardiovascular changes in response to brief complete cessations of uterine blood flow are reflexly mediated, since they can be completely abolished by denervation of the carotid and aortic chemoreceptors.

The tachycardia that we observed during the latter part of the period of reduced uterine blood flow continued for up to 2 hours after the release of the vascular clamp, when fetal arterial blood gases had returned to near normal levels. A prolonged tachycardia has also been described following 60-minute episodes of fetal hypoxemia induced by maternal hypoxemia.<sup>10,13</sup> Because this tachycardia can be abolished by treatment with a  $\beta$ -adrenergic blocking agent, propranolol,<sup>13</sup> it is likely that the tachycardia observed in our experiments is a consequence of a prolonged increase in sympathetic drive and may be important for maintaining umbilical blood flow. It is possible that the tachycardia may be

secondary to a prolonged increase in circulating catecholamine concentrations in the fetus, since maternal hypoxemia is also known to elevate fetal catecholamine concentrations.<sup>13, 15</sup> Catecholamine concentrations, however, have usually returned to normal levels by 120 minutes after the end of the hypoxic episode.<sup>13, 15</sup> This was a time when our fetuses were still exhibiting a prolonged tachycardia.

In the human fetus the prolonged absence of accelerations in antepartum fetal heart rate is associated with fetal acidosis and an increase in both perinatal morbidity and mortality.<sup>1, 2</sup> It has been suggested that the appearance of decelerations in fetal heart rate following uterine contractions is an earlier sign of fetal hypoxemia than the absence of accelerations in the rhesus monkey.<sup>16</sup> This study of the characteristics of accelerations and decelerations in heart rate of fetal sheep during the latter third of gestation demonstrates that the cardiovascular responses to prolonged reductions in uterine blood flow and its associated mild hypoxemia are tachycardia and an increase in the number of decelerations. Although we do not suggest that this information can be directly related to the interpretation of human antepartum fetal heart rate recordings, it is only through a careful quantitative analysis of the cardiovascular changes during controlled hypoxemia that we may begin to understand the pathophysiology of these indirect signs of fetal health that are commonly used in clinical practice.

We wish to thank Miss J. Buttress and Miss J. Norman for their excellent technical assistance and Mr. N. Yanios for his assistance in writing the heart rate analysis program. We are also grateful to Dr. D. W. Walker and Prof. G. D. Thorburn for their interest in this work.

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# Inhibition of human chorionic gonadotropin production by prolactin from term human trophoblast

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In vivo suppression of prolactin concentrations by bromocriptine near term, in a pregnant woman with a prolactinoma, was followed by augmentation of human chorionic gonadotropin levels. Suspension of drug therapy at 38 weeks of gestation was followed by a reversal of this sequence of events. In vitro, both ovine prolactin and human prolactin added to explants of term placental trophoblast significantly inhibited human chorionic gonadotropin production from this tissue. Although, with ovine prolactin, this inhibitory effect was demonstrable up to 5 µg/ml of ovine prolactin in the culture medium, only doses of 0.1 to 0.2 µg/ml of human prolactin significantly suppressed human chorionic gonadotropin production. Overall, 0.1 and 0.2 µg/ml of ovine prolactin and human prolactin most consistently suppressed human chorionic gonadotropin production to a statistically significant extent. In general, the concentrations in the culture medium of both ovine prolactin and human prolactin that inhibited human chorionic gonadotropin production in vitro were comparable to the concentrations of prolactin present in the mother and fetus. These in vivo and in vitro observations suggest that prolactin inhibits human chorionic gonadotropin production from term human trophoblast. (AM J OBSTET GYNECOL 1986;154:336-40.)

**Key words:** Chorionic gonadotropin, human; prolactin, human; prolactin, ovine; bromocriptine

The mechanisms regulating production of human chorionic gonadotropin (hCG) from the placenta have not been fully elucidated. We have previously presented evidence suggesting that prolactin may play a role in the control of hCG production during human pregnancy near term.<sup>1</sup> Lowering of plasma prolactin levels by bromocriptine therapy in a patient with a prolactin-secreting pituitary adenoma resulted in augmentation of the plasma hCG levels during late but not early pregnancy. This sequence of events was reversed subsequent to the suspension of treatment near delivery. Furthermore, there is a significant negative correlation in the plasma levels of hCG and prolactin in maternal blood throughout pregnancy.<sup>1</sup> These in vivo findings suggested that, during late pregnancy, prolactin and hCG interacted in a manner by which the production of hCG by term trophoblast may be inhibited by prolactin. Studies in vitro also suggest other controlling mechanisms of hCG production in trophoblast ob-

tained from term placentas. Gonadotropin-releasing hormone stimulates<sup>2</sup> while progesterone inhibits hCG production from this tissue.<sup>3</sup>

We now present additional data, from both in vivo and in vitro observations, that provide further evidence of an inhibitory effect of prolactin on hCG release from the human placenta at term.

## Material and methods

**In vivo observations.** Patient D. M. (aged 35 years) had secondary infertility due to anovulation resulting from hyperprolactinemia, which was caused by a prolactinoma. She experienced the galactorrhea-amenorrhea syndrome for 11 years before conceiving the current pregnancy. Treatment with bromocriptine in progressively increasing doses up to 5 mg in two divided doses successfully lowered the prolactin levels. Thereafter, ovulation was restored and was followed by conception. The pregnancy was uneventful until the third trimester when moderate to severe headaches and rising prolactin levels (Fig. 1) suggested that the pituitary tumor was expanding. Accordingly, treatment with bromocriptine was restarted in dosages as shown in Fig. 1. Plasma samples were obtained for determination of prolactin and hCG levels at intervals of once or twice weekly until term. Visual field examinations showed no abnormalities. Symptoms subsided and after spontaneous labor she was delivered of a normal male infant. She successfully breast-fed the infant without complications.

**In vitro experiments.** In these experiments, ovine

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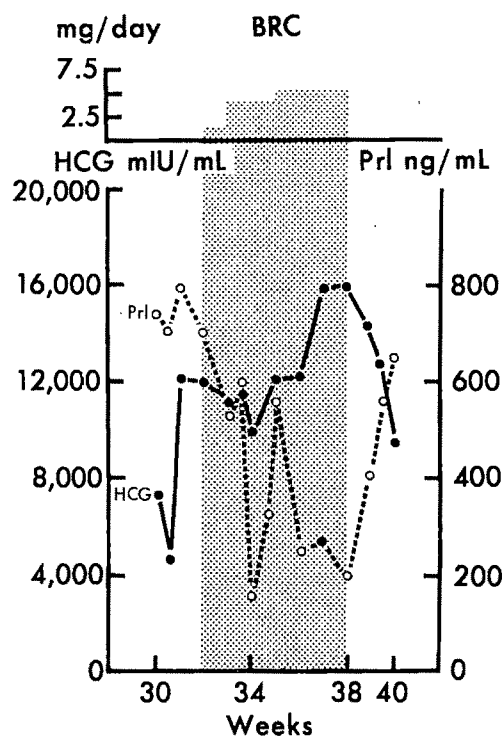
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and human prolactin were added separately to explants of trophoblastic tissue. Placental tissue was obtained from four healthy patients undergoing repeat elective cesarean section between 38 and 40 weeks of gestation. Trophoblast was isolated by careful removal of the fetal membranes and decidual tissue under sterile conditions immediately after delivery of the placenta. Placental tissues were washed 2 or 3 times with culture medium to remove blood and blood clots. Thereafter, the trophoblast was minced with a scalpel blade to pieces of approximately 1 to 2 mm<sup>3</sup>, washed another three times with the medium in a clinical centrifuge at 800 rpm, and divided among Falcon plastic Petri dishes. The explants were then incubated in triplicate in Eagle's minimum essential medium with 5% fetal calf serum (Grand Island Biological Company), 0.1 mmol of non-essential amino acids, 2 mmol of L-glutamine, 100 units/ml of penicillin, 0.25 µg/ml of fungizone, and 100 µg/ml of streptomycin at 36° C under an atmosphere of 5% carbon dioxide in air. Control culture dishes contained no added prolactin. In two experiments with ovine prolactin, doses of 0.2, 1 and 5 µg/ml of culture medium were used in triplicate dishes and cumulative hCG production was expressed per milligram of protein. In two experiments, human prolactin was used in concentrations ranging between 0.05, 0.1, 0.2, 1, and 5 µg/ml of culture medium and between 3 and 5 culture dishes were used for each dose level. The cumulative amount of hCG produced in each culture was expressed per milligram of protein in one experiment and per gram weight of tissue in another. Media were harvested at various intervals as shown in Figs. 2 through 5 and frozen at -20° C until the β-subunit of hCG was assayed by means of a double-antibody radioimmunoassay with kits purchased from BIO-RIA, Montreal, Quebec, Canada. At the termination of each experiment, the explants were washed twice with 0.9% saline solution by centrifugation at 800 rpm. Protein contents of the tissues were then determined by the method of Lowry et al.<sup>4</sup> after treatment with 1N sodium hydroxide.

Highly purified human prolactin and ovine prolactin (oPRL-14) were generous gifts from Dr. Henry Friesen, University of Manitoba, and the National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases, Bethesda, Maryland, respectively. Data were analyzed by the *t* test.

## Results

**In vivo observations.** As shown in Fig. 1, by 30 weeks of gestation prolactin levels reached 790 ng/ml. After reinitiation of bromocriptine therapy at 32 weeks of gestation in gradually increasing doses, prolactin levels declined. The patient's headache was also relieved. During this interval hCG concentrations rose with the highest level occurring at the nadir of the prolactin



**Fig. 1.** Plasma prolactin (Prl) and hCG levels (β-subunit assay) during the third trimester of pregnancy in a patient with a known prolactinoma in whom symptoms of tumor expansion resulted in reinitiation of treatment with bromocriptine (BRC) as shown. The results are expressed as milli-International Units of the second international standard.

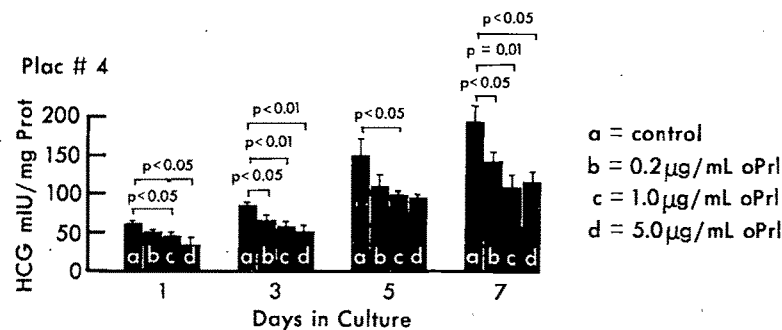
levels at 38 weeks when bromocriptine was withdrawn. Concomitant with the subsequent rise in prolactin levels, there was a consistent decline in hCG levels until term. Following the discontinuation of medication the patient remained free of headaches.

## In vitro experiments (n = 4)

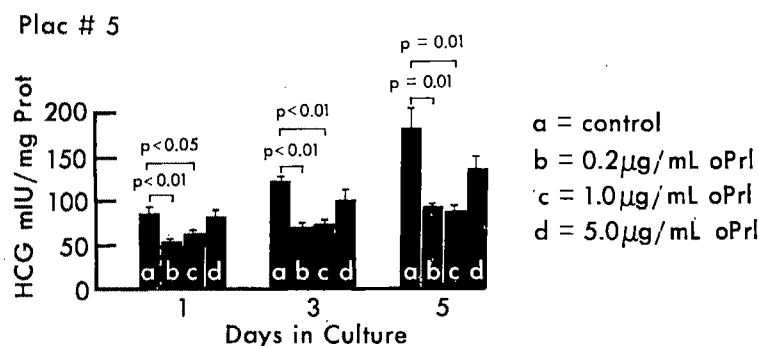
### Experiments with ovine prolactin

**EXPERIMENT 1 (PLACENTA 4).** As shown in Fig. 2, all cultures containing ovine prolactin had decreased hCG production when compared with control cultures. On day 1 the cultures with 1 and 5 µg/ml of ovine prolactin had significantly lower hCG values. By day 3, cultures of all dose levels of ovine prolactin tested had significantly lower hCG values as compared with control cultures. A similar trend was maintained by day 5; however, only the culture with a dose level of 1 µg/ml of ovine prolactin produced a value that was significantly lower than the control value. At day 7, all cultures of ovine prolactin tested exhibited significantly lower hCG production as compared with control cultures.

**EXPERIMENT 2 (PLACENTA 5).** As shown in Fig. 3, cultures containing 0.2 and 1 µg/ml of ovine prolactin significantly suppressed the release of hCG into the medium throughout the experiment. Although 5 µg/ml of ovine prolactin did not cause a statistically significant reduction of hCG production, mean levels were



**Fig. 2.** The effect of exogenous ovine prolactin (oPrI) on the production of hCG by placental explants is shown. hCG was measured by the  $\beta$ -subunit of hCG radioimmunoassay, in milli-International Units of the second international standard. The data are expressed on a per milligram of protein basis. Control cultures contained no added ovine prolactin. The dose of ovine prolactin is shown (micrograms per milliliter of culture medium). Data are shown as mean  $\pm$  SEM of three cultures.



**Fig. 3.** The effect of exogenous ovine prolactin (oPrI) on the production of hCG by placental explants is shown. hCG was measured by the  $\beta$ -subunit of hCG radioimmunoassay in milli-International Units of the second international standard. The data are expressed on a per milligram of protein basis. Control cultures contained no added ovine prolactin. The dose of ovine prolactin is shown (micrograms per milliliter of culture medium). Data are shown as mean  $\pm$  SEM of three cultures.

lower in all cultures containing ovine prolactin, especially at days 3 and 5.

#### Experiments with human prolactin

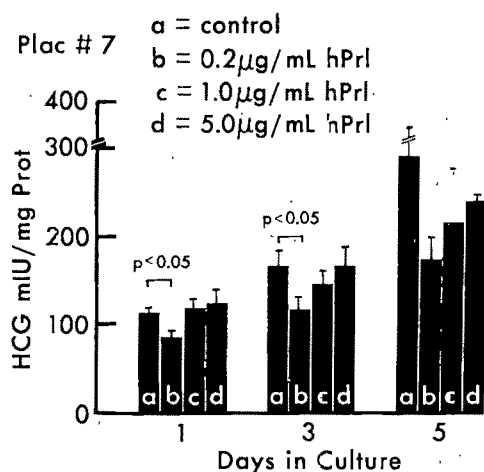
**EXPERIMENT 1 (PLACENTA 7).** As shown in Fig. 4, doses of human prolactin used in this experiment were identical to those used in the two experiments with ovine prolactin. The dose of 0.2 µg/ml of human prolactin significantly suppressed hCG production on days 1 and 3 of the experiment. By day 3, the cultures with 1 µg/ml of human prolactin had slightly lower hCG production, while cultures with the 5 µg/ml dose produced values similar to those of controls. By day 5, all human prolactin doses tested produced lower levels of hCG production as compared to control cultures, but the differences did not reach statistical significance.

**EXPERIMENT 2 (PLACENTA 8).** As shown in Fig. 5, the dose of human prolactin in this experiment included 0.05 and 0.1 µg/ml in addition to 0.2, 1.0 and 5 µg/ml of human prolactin that were used in the two experiments with ovine prolactin and the one other experiment with human prolactin. The 0.1 µg/ml dose of

human prolactin significantly suppressed hCG production throughout the experiment while all other dose levels of human prolactin showed no significantly different effects on hCG production when compared with those of controls.

#### Comment

**In vivo observations.** In this patient, with a prolactinoma that was possibly expanding, reinitiation of bromocriptine therapy in the third trimester of pregnancy relieved headache and inhibited prolactin secretion. During treatment with bromocriptine, the hCG levels rose while prolactin levels declined. With the suspension of drug therapy at 38 weeks of gestation, the subsequent increase in prolactin levels was associated with a decline of hCG levels to term, when delivery occurred (Fig. 1). These findings confirm our previous in vivo observations in another patient with a prolactinoma treated with bromocriptine during pregnancy.<sup>1</sup> In this patient, the decline in prolactin levels induced by bromocriptine also resulted in enhanced hCG levels. On



**Fig. 4.** The effect of exogenous human prolactin (hPrL) on the production of hCG by placental explants is shown. hCG was measured by the  $\beta$ -subunit of hCG radioimmunoassay in milli-International units of the second international standard. The data are expressed on a per milligram of protein basis. Control cultures contained no added human prolactin. The dose of human prolactin (micrograms per milliliter of culture medium) added to the remaining dishes is shown. Data are shown as mean  $\pm$  SEM of three cultures.

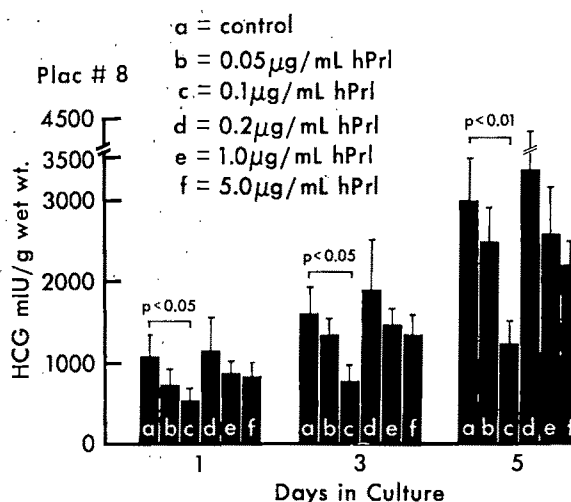
suspension of treatment with bromocriptine, the rising levels of prolactin were associated with concomitant inhibition of hCG levels.

#### In vitro experiments

**Experiments with ovine prolactin.** In the two experiments with ovine prolactin (Figs. 2 and 3), dose levels between 0.1 and 5  $\mu$ g/ml inhibited hCG production by explants of human trophoblastic tissue from term placenta. However, in contrast to experiment 1 (Fig. 2), in experiment 2 (Fig. 3) the production of hCG, although lower in cultures containing 5  $\mu$ g/ml of ovine prolactin than in control cultures, did not reach statistical significance.

**Experiments with human prolactin.** In the first experiment with human prolactin (Fig. 4) 0.2  $\mu$ g/ml suppressed hCG production in a consistent fashion throughout the experiment, except on day 5, when the production of hCG, although lowest at this dose, this did not reach statistical significance. Nevertheless, by day 5, all cultures containing human prolactin produced less hCG. In the second experiment with human prolactin (Fig. 5), reduced hCG production was observed in cultures containing 0.1  $\mu$ g/ml of human prolactin in the culture medium.

Taken together, the current data derived from both in vivo and in vitro observations are consistent with our previous in vivo studies suggesting that prolactin suppresses hCG production from the placenta near term.<sup>1</sup> These in vivo observations are confirmed by the current observations in the patient with a prolactinoma experiencing symptoms of tumor expansion in late preg-



**Fig. 5.** The effect of exogenous human prolactin of hCG by placental explants is shown. hCG was measured by the  $\beta$ -subunit of hCG radioimmunoassay, in milli-International Units of the second international standard. The data are expressed on a per gram of tissue wet weight basis. Control cultures contained no human prolactin. The dose of human prolactin is shown (micrograms per milliliter of culture medium). Data are shown as mean  $\pm$  SEM of five cultures.

nancy (Fig. 1). As we previously observed,<sup>1</sup> treatment with bromocriptine resulted in suppression of prolactin levels and augmentation of hCG concentrations, and a reversal of these events occurred once treatment was withdrawn. These observations are extended by our current in vitro data (Figs. 2 through 5) in which we demonstrate that both human and ovine prolactin may suppress the production of hCG from explants of term trophoblastic tissue. This suppressive effect extends up to 5  $\mu$ g of prolactin per milliliter of culture medium. However, when we consider both the human and ovine species of prolactin studied, the 0.1 and 0.2  $\mu$ g/ml dose levels in the culture medium were most consistent in suppressing hCG production, to a statistically significant extent.

The observations that the higher doses of prolactin (1 to 5  $\mu$ g/ml) used in the in vitro experiments were less consistent in inhibiting hCG production from the trophoblast conform with several other effects of prolactin. The response of hCG production from the placental explants to various concentrations of prolactin approximates the "bell-shaped" dose-response curve.<sup>7</sup> Several examples where certain effects of prolactin that are demonstrable at one concentration but then disappear (or are reversed) at higher concentrations are also documented.<sup>7</sup> Suppression of prolactin concentrations by bromocriptine resulted in reduced progesterone production during the luteal phase of the menstrual cycle.<sup>8</sup> Reduction of progesterone levels during the luteal phase was also observed when prolactin concentrations were elevated by sulpiride.<sup>8</sup> Other evidence



indicates that high concentrations of prolactin inhibit progesterone secretion in the culture of human granulosa cells.<sup>9</sup> Concentrations of prolactin corresponding to the physiologic range in blood appear necessary for optimum progesterone production from these cells. Neutralization of prolactin with prolactin antisera added to the culture medium inhibited progesterone production from granulosa cells.<sup>9</sup> Prolactin has also been shown to inhibit progesterone production from small (immature) ovarian follicles while it stimulated progesterone production from larger (mature) follicles.<sup>10</sup> Although the mechanism of prolactin action has yet to be fully elucidated, recent experiments have provided some insights into these responses to the hormone. Kelly et al.<sup>11</sup> have demonstrated that prolactin not only induces its own receptors ("up-regulation") but also is capable of producing an inhibitory effect on these receptors ("down-regulation"). Prolactin administration resulted in a decrease in its receptors in the mammary glands of rabbits for up to 1 to 6 hours with a return to baseline levels by 30 hours. In organ culture, this down-regulation of prolactin receptors can be observed with the presence of 1 µg/ml of prolactin in the culture medium.<sup>11</sup> The interaction of prolactin with its receptors in mammary tissue initiates a sequence of events resulting in synthesis of milk proteins. The response of rabbit mammary tissue to prolactin also conforms to the "bell-shaped" dose-response curve noted previously. Peak casein synthesis occurred between 0.1 and 1 µg/ml of prolactin. As compared with these doses, the doses of prolactin above 1 and up to 20 µg/ml inhibited casein synthesis. Near-maximal receptor down-regulation occurred at the 1 µg/ml dose level. Our results on the effects of prolactin on the production of hCG from explants of term human trophoblast appear consistent with these and the other known responses to prolactin.<sup>7-11</sup> Since dopamine inhibited hCG production from explants of trophoblast,<sup>12</sup> the effects on prolactin and hCG concentrations we observed in vivo with bromocriptine therapy (Fig. 1 and reference 1) cannot be explained on the basis of a direct dopaminergic effect of the drug.

In general, our present data, based on both in vivo and in vitro observations, suggest that prolactin exerts an inhibitory effect on hCG production from term trophoblast. This effect is demonstrable in vitro with the use of concentrations of prolactin normally present in the maternal-fetal unit.<sup>1,5,6</sup> In view of the close anatomic relationship of the trophoblast with the decidua, which synthesizes prolactin de novo,<sup>13</sup> the possibility of a synergistic effect between decidual prolactin and hCG from trophoblast near term cannot be ignored. That such a possibility may exist is supported by the novel observations of Rosenberg and Bhatnagar<sup>14</sup> indicating that hCG itself could stimulate decidual prolactin se-

cretion. Decidual prolactin secretion is not affected by bromocriptine and dopamine in vitro.<sup>15</sup> In contrast, during late pregnancy, bromocriptine can influence both prolactin and hCG concentrations in the maternal compartment. Collectively, these observations raise the possibility that an extradecidual source of prolactin (for example, the maternal pituitary) contributes, at least in part, to the control of hCG secretion from the near-term placenta.

We thank Mr. A. Duleba, Mrs. L. Sy, Mrs. T. Yang, Miss P. Davis, and Mrs. P. Burch Callegari for their assistance.

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# Less withdrawal bleeding: another reason for OGEN<sup>®</sup> (estropipate)



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**Percentage of women without withdrawal bleeding, taking either OGEN 1.25 or conjugated estrogens 0.625 mg<sup>1</sup>**

OGEN <sup>®</sup> 1.25	42% (19/45)
conjugated estrogens 0.625	18% (9/50)
<i>p</i> < 0.05.      Adapted from Wren and Routledge, 1982. <sup>1</sup>	

\*Both regimens were given 24 days out of 30, with levonorgestrel 0.03 mg, a progestogen, added from the 15th to 24th day.

## OGEN<sup>®</sup> (estropipate)

*Only estrone. Identical to the body's own.*





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**Reference:**

I. Wren BG, Brown LB, Routledge DA: Differential clinical response to oestrogens after menopause. *Med J Aust* 2:329-332, 1982.

**OGEN<sup>®</sup>**  
(estropipate)

*Only estrone. Identical to the body's own.*





# **OGEN®** ESTROPIRATE TABLETS, USP Tablets

## **WARNING:**

### **1. ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA.**

Three independent case control studies have shown an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for prolonged periods. This risk was independent of the other known risk factors for endometrial cancer. These studies are further supported by the finding that incidence rates of endometrial cancer have increased sharply since 1959 in eight different areas of the United States with population-based cancer reporting systems, an increase which may be related to the rapidly expanding use of estrogens during the last decade.

The three case control studies reported that the risk of endometrial cancer in estrogen users was about 4.5 to 13.9 times greater than in nonusers. The risk appears to depend on both duration of treatment and on estrogen dose. In view of these findings, when estrogens are used for the treatment of menopausal symptoms, the lowest dose that will control symptoms should be utilized and medication should be discontinued as soon as possible. When prolonged treatment is medically indicated, the patient should be reassessed on at least a semiannual basis to determine the need for continued therapy. Although the evidence must be considered preliminary, one study suggests that cyclic administration of low doses of estrogen may carry less risk than continuous administration; it therefore appears prudent to utilize such a regimen.

Close clinical surveillance of all women taking estrogens is important. In all cases of undiagnosed persistent or recurring abnormal vaginal bleeding, adequate diagnostic measures should be undertaken to rule out malignancy.

There is no evidence at present that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equieffective doses.

### **2. OGEN SHOULD NOT BE USED DURING PREGNANCY.**

According to some investigators, the use of female sex hormones, both estrogens and progestogens, during early pregnancy may seriously damage the offspring. Studies have reported that females exposed in utero to diethylstilbestrol, a non-steroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. In one of these studies, this risk was estimated as not greater than 4 per 1000 exposures. Furthermore, there are reports that a high percentage of such exposed women (from 30 to 60 percent) have been found to have vaginal adenosis, epithelial changes of the vagina and cervix. Although these reported changes are histologically benign, the investigators have not determined whether they are precursors of adenocarcinoma.

Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies in the offspring, including heart defects and limb reduction defects. One case control study estimated a 4.7 fold increased risk of limb reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 per 1000.

In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. OGEN has not been studied for these uses, and therefore should not be used during pregnancy. There is no evidence from well controlled studies that progestogens are effective for these uses.

If OGEN (estropipate tablets) is used during pregnancy, or if the patient becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus, and the question of continuation of the pregnancy should be addressed.

## **INDICATIONS AND USAGE**

The cyclic administration (See "DOSAGE AND ADMINISTRATION" section) of OGEN (estropipate tablets) is indicated for the treatment of estrogen deficiency associated with:

1. Moderate to severe vasomotor symptoms of menopause. (There is no evidence that estrogens are effective for nervous symptoms or depression which might occur during menopause, and they should not be used to treat these conditions.)
2. Atrophic vaginitis.
3. Kraurosis vulvae.
4. Female hypogonadism.
5. Female castration.
6. Primary ovarian failure.

**OGEN (ESTROPIRATE TABLETS) HAS NOT BEEN TESTED FOR EFFICACY FOR ANY PURPOSE DURING PREGNANCY. SINCE ITS EFFECT UPON THE FETUS IS UNKNOWN, IT CANNOT BE RECOMMENDED FOR ANY CONDITION DURING PREGNANCY (SEE BOXED WARNING).**

## **CONTRAINDICATIONS**

OGEN should not be used in women with any of the following conditions:

1. Known or suspected cancer of the breast.
2. Known or suspected estrogen-dependent neoplasia.
3. OGEN may cause fetal harm when administered to a pregnant woman. OGEN is contraindicated in women who are or may become pregnant (See Boxed Warning).
4. Undiagnosed abnormal genital bleeding.
5. Active thrombophlebitis or thromboembolic disorders.
6. A past history of thrombophlebitis, thrombosis, or thromboembolic disorders associated with previous estrogen use.

## **WARNINGS**

**1. Induction of malignant neoplasms.** Long-term continuous administration of natural and synthetic estrogens in certain animal species has been reported by some investigators to increase the frequency of carcinomas of the breast, cervix, vagina, and liver. There is now evidence that estrogens increase the risk of carcinoma of the endometrium in humans. (See Boxed Warning).

At the present time there is no conclusive evidence that estrogens given to postmenopausal women increase the risk of cancer of the breast. There are, however, a few retrospective studies which suggest a small but statistically significant increase in the risk factor for breast cancer among these women. Therefore, caution should be exercised when administering estrogens to women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease, or abnormal mammograms. Careful breast examinations should be performed periodically.

**2. Gall bladder disease.** A recent study has reported a 2 to 3-fold increase in the risk of surgically confirmed gall bladder disease in women receiving postmenopausal estrogens, similar to the 2-fold increase previously noted in users of oral contraceptives. In the case of oral contraceptives, the increased risk appeared after two years of use.

**3. Effects similar to those caused by estrogen-progestogen oral contraceptives.** There are several serious adverse effects of oral contraceptives, most of which have not, up to now, been documented as consequences of postmenopausal estrogen therapy. This may reflect the comparatively low doses of estrogen used in postmenopausal women. It would

be expected that the larger doses of estrogen used to treat postpartum breast engorgement would be more likely to result in these adverse effects, and, in fact, it has been shown that there is an increased risk of thrombosis in women receiving estrogens for postpartum breast engorgement.

**a. Thromboembolic disease.** It is now well established that users of oral contraceptives have an increased risk of various thromboembolic and thrombotic vascular diseases, such as thrombophlebitis, pulmonary embolism, stroke, and myocardial infarction. Cases of retinal thrombosis, mesenteric thrombosis, and optic neuritis have been reported in oral contraceptive users. There is evidence that the risk of several of these adverse reactions is related to the dose of the drug. An increased risk of post-surgery thromboembolic complications has also been reported in users of oral contraceptives. If feasible, estrogen should be discontinued at least 4 weeks before surgery of the type associated with an increased risk of thromboembolism; it should also be discontinued during periods of prolonged immobilization.

While an increased rate of thromboembolic and thrombotic disease in postmenopausal users of estrogens has not been found this does not rule out the possibility that such an increase may be present or that subgroups of women who have underlying risk factors or who are receiving relatively large doses of estrogens may have increased risk. Therefore estrogens should not be used in persons with active thrombophlebitis or thromboembolic disorders, and they should not be used in persons with a history of such disorders in association with estrogen use. They should be used with caution in patients with cerebral vascular or coronary artery disease and only for those in whom estrogens are clearly needed.

Large doses of estrogen (5 mg conjugated estrogens per day), comparable to those used to treat cancer of the prostate and breast, have been shown in a large prospective clinical trial in men to increase the risk of nonfatal myocardial infarction, pulmonary embolism and thrombophlebitis. When estrogen doses of this size are used, any of the thromboembolic and thrombotic adverse effects associated with oral contraceptive use should be considered a clear risk.

**b. Hepatic adenoma.** Benign hepatic adenomas appear to be associated with the use of oral contraceptives. Although benign, and rare, these may rupture and cause death through intraabdominal hemorrhage. Such lesions have not yet been reported in association with other estrogen or progestogen preparations but should be considered in estrogen users having abdominal pain and tenderness, abdominal mass, or hypovolemic shock. Hepatocellular carcinoma has also been reported in women taking estrogen-containing oral contraceptives. The relationship of this malignancy to these drugs is not known at this time.

**c. Elevated blood pressure.** Increased blood pressure is not uncommon in women using oral contraceptives. There is now a report that this may occur with use of estrogens in the menopause and blood pressure should be monitored with estrogen use, especially if high doses are used.

**d. Glucose tolerance.** A worsening of glucose tolerance has been observed in a significant percentage of patients on estrogen-containing oral contraceptives. For this reason, diabetic patients should be carefully observed while receiving estrogen.

**e. Hypercalcemia.** Administration of estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases. If this occurs, the drug should be stopped and appropriate measures taken to reduce the serum calcium level.

## **PRECAUTIONS**

### **A. General Precautions.**

1. A complete medical and family history should be taken prior to the initiation of an estrogen therapy. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen, and pelvic organs, and should include a Papanicolaou smear. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination being performed.

2. Fluid retention — Estrogens may cause some degree of fluid retention. Therefore, patients with conditions such as epilepsy, migraine, and cardiac or renal dysfunction, which might be influenced by this factor, require careful observation.

3. Certain patients may develop undesirable manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding, mastodynia, etc.

4. Oral contraceptives appear to be associated with an increased incidence of mental depression. Although it is not clear whether this is due to the estrogenic or progestogenic component of the contraceptive, patients with a history of depression should be carefully observed.

5. Preexisting uterine leiomyomata may increase in size during estrogen use.

6. The pathologist should be advised of the patient's use of estrogen therapy when relevant specimens are submitted.

7. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving estrogen-containing oral contraceptive therapy. If jaundice develops in any patient receiving estrogen, the medication should be discontinued while the cause is investigated.

8. Estrogens may be poorly metabolized in patients with impaired liver function and they should be administered with caution in such patients.

9. Because estrogens influence the metabolism of calcium and phosphorus, they should be used with caution in patients with metabolic bone diseases that are associated with hypercalcemia or in patients with renal insufficiency.

**B. Information for the Patient.** See text of Patient Package Insert which appears after PHARMACIAN REFERENCES.

**C. Drug Interactions.** The concomitant use of any drugs which can induce hepatic microsomal enzymes with estrogens may produce estrogen levels which are lower than would be expected from the dose of estrogen administered.

The use of broad spectrum antibiotics which profoundly affect intestinal flora may influence the absorption of steroidal compounds including the estrogens.

Diabetics receiving insulin may have increased insulin requirements when receiving estrogens.

Laboratory Test Interference. Certain endocrine and liver function tests may be affected by estrogen-containing oral contraceptives. The following similar changes may be expected with larger doses of estrogen:

- a. Increased sulfobromophthalen retention
- b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin III; increased norepinephrine-induced platelet aggregability.
- c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by PBI, T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered.
- d. Abnormal glucose tolerance test results.
- e. Decreased pregnandiol excretion.
- f. Reduced response to metyrapone test.
- g. Reduced serum folate concentration.
- h. Increased serum triglyceride and phospholipid concentration.

**D. Carcinogenesis.** Studies have shown an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for prolonged periods (See Boxed Warning). At the present time there is no conclusive evidence that estrogens given to postmenopausal women increase the risk of cancer of the breast. There are, however, a few retrospective studies which suggest a small but statistically significant increase in the risk factor for breast cancer among these women. (See "WARNINGS" section.)

**E. Pregnancy.** Pregnancy Category X. See "CONTRAINDICATIONS" section and Boxed Warning.

**F. Nursing Mothers.** Estrogens have been reported to be excreted in human breast milk. Caution should be exercised when OGEN is administered to a nursing woman.

**G. Pediatric Use.** Because of the effects of estrogens on epiphyseal closure, they should be used judiciously in young patients in whom bone growth is not complete.

## **ADVERSE REACTIONS**

(See Warnings regarding reports of possible induction of neoplasia, unknown effects upon the fetus, increased incidence of gall bladder disease, and adverse effects similar to those of oral contraceptives, including thromboembolism.) The following additional adverse reactions in decreasing order of severity within each category have been reported with estrogenic therapy, including oral contraceptives:

1. **Genitourinary system.**  
Increase in size of uterine fibromyomata.  
Vaginal candidiasis.  
Cystitis-like syndrome.  
Dysmenorrhea.  
Amenorrhea during and after treatment.  
Change in cervical eversion and in degree of cervical secretion.  
Breakthrough bleeding, spotting, change in menstrual flow.  
Premenstrual-like syndrome.
2. **Breast.**  
Tenderness, enlargement, secretion.
3. **Gastrointestinal.**  
Cholestatic jaundice.  
Nausea, vomiting.  
Abdominal cramps, bloating.
4. **Skin.**  
Hemorrhagic eruption.  
Erythema nodosum.  
Erythema multiforme.  
Hirsutism.  
Chloasma or melasma which may persist when drug is discontinued.  
Loss of scalp hair.
5. **Eyes.**  
Steepening of corneal curvature.  
Intolerance to contact lenses.
6. **CNS.**  
Chorea.  
Mental depression.  
Migraine, dizziness, headache.
7. **Miscellaneous.**  
Aggravation of porphyria.  
Edema.  
Reduced carbohydrate tolerance.  
Increase or decrease in weight.  
Changes in libido.

## **OVERDOSAGE**

Numerous reports of ingestion of large doses of estrogen-containing oral contraceptives by young children indicate that serious ill effects do not occur. Overdosage of estrogen may cause nausea and withdrawal bleeding may occur in females.

## **DOSAGE AND ADMINISTRATION**

### **1. Given cyclically for short-term use:**

For treatment of moderate to severe vasomotor symptoms, atrophic vaginitis, or kraurosis vulvae associated with the menopause.

The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible.

Administration should be cyclic (e.g., 3 weeks on and 1 week off).

Attempts to discontinue or taper medication should be made at 3 to 6 month intervals.

Usual dosage ranges:

**Vasomotor symptoms** — One OGEN .625 Tablet to one OGEN 5 Tablet per day. The lowest dose that will control symptoms should be chosen. If the patient has not menstruated within the last two months or more, cyclic administration is started arbitrarily. If the patient is menstruating, cyclic administration is started on day 5 of bleeding.

**Atrophic vaginitis and kraurosis vulvae** — One OGEN .625 Tablet to one OGEN 5 Tablet daily, depending upon the tissue response of the individual patient. The lowest dose that will control symptoms should be chosen. Administer cyclically.

### **2. Given cyclically:**

Female hypogonadism; female castration; primary ovarian failure.

Usual dosage ranges:

**Female hypogonadism** — A daily dose of one OGEN 1.25 Tablet to three OGEN 2.5 Tablets may be given for the first three weeks of a theoretical cycle, followed by a rest period of eight to ten days. The lowest dose that will control symptoms should be chosen. If bleeding does not occur by the end of this period, the same dosage schedule is repeated. The number of courses of estrogen therapy necessary to produce bleeding may vary depending on the responsiveness of the endometrium. If satisfactory withdrawal bleeding does not occur, an oral progestogen may be given in addition to estrogen during the third week of the cycle.

**Female castration and primary ovarian failure** — A daily dose of one OGEN 1.25 Tablet to three OGEN 2.5 Tablets may be given for the first three weeks of a theoretical cycle, followed by a rest period of eight to ten days. Adjust dosage upward or downward according to severity of symptoms and response of the patient. For maintenance, adjust dosage to lowest level that will provide effective control.

Treated patients with an intact uterus should be monitored closely for signs of endometrial cancer and appropriate diagnostic measures should be taken to rule out malignancy in the event of persistent or recurring abnormal vaginal bleeding.

## **HOW SUPPLIED**

OGEN (estropipate tablets, USP) is supplied as OGEN .625 (0.75 mg estropipate), yellow tablets, NDC 0074-3943-04; OGEN 1.25 (1.5 mg estropipate), peach-colored tablets, NDC 0074-3946-04; OGEN 2.5 (3 mg estropipate), blue tablets, NDC 0074-3951-04; and OGEN 5 (6 mg estropipate), light green tablets, NDC 0074-3958-13. Tablets of all four dosage levels are standardized to provide uniform estrone activity and are grooved (Divide-Tab®) to provide dosage flexibility. All tablet sizes of OGEN are available in bottles of 100.

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# The influence of prolonged pregnancy on infant development at one and two years of age: A prospective controlled study

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Isolated reports of developmental disturbances following prolonged pregnancy led us to compare, prospectively, at 1 and 2 years of age, infants born after normal term gestations with those born after prolonged pregnancies (exceeding 294 days). The infants were subgrouped according to their physical condition at birth, that is, normal or dysmature (mild or advanced dysmaturity). Infant assessments included: (1) height and weight, (2) hospitalizations, and (3) mental development by the Griffiths Mental Development Scales. Follow-up testing was obtained on 130 term control infants and 89 infants of prolonged pregnancies at 1 year of age and 111 term control infants and 76 infants of prolonged pregnancies at 2 years of age. At 1 and 2 years the general intelligence quotient, physical milestones, and intercurrent illnesses for normal infants and those of prolonged pregnancies were not significantly different. (AM J OBSTET GYNECOL 1986;154:341-5.)

**Key words:** Prolonged pregnancy, postterm pregnancy, development, dysmaturity, postmaturity

The management of prolonged pregnancy (exceeding 294 days), a common problem faced by obstetricians, remains a controversial issue. Decision making, as with all aspects of obstetrics, must take into account both perinatal and long-term sequelae.

Prolonged pregnancy has been associated with a two-fold to sevenfold increase in perinatal morbidity and mortality.<sup>1,2</sup> This increased risk appears to be primarily associated with fetal dysmaturity (postmaturity). The majority of postterm fetuses, however, are neither dysmature nor hypoxic and can be expected to have an uncomplicated perinatal course.<sup>3,4</sup>

Few investigators have addressed the question of long-term outcome for postterm infants. Isolated reports have suggested an increase in morbidity, and lower developmental testing scores among the dysmature group.<sup>5-7</sup> In addition, the postterm eutrophic infant may be at risk for long-term sequelae.<sup>8</sup>

The present study was undertaken to determine the extent and frequency (if any) of developmental disturbances associated with prolonged pregnancy. We were

interested in knowing whether dysmaturity carries special risks not seen in the eutrophic neonate. We were also interested in knowing whether prolonged pregnancy per se (even in the absence of dysmaturity) carries long-term risks that might reflect lesser degrees of fetal hypoxia from subclinical placental insufficiency.

## Subjects and methods

From 1981 to 1983, 184 term control patients and 129 cases of prolonged pregnancy (exceeding 294 days) were entered into the study. All patients were delivered of their infants at Toronto General Hospital. The patients were entered into the study within 1 week of the expected date of confinement of an uncomplicated pregnancy. The criteria were as follows: (1) a normal last menstrual period, where the date of onset was certain and the cycles were regular and monthly; (2) an ultrasound examination before 20 weeks, which agreed with the menstrual dates; and (3) no history of recent oral contraceptive usage, amenorrhea, irregular menses, or nursing when the pregnancy occurred. Those pregnancies extending beyond 294 completed days were considered prolonged, and the others became control pregnancies.

Perinatal data were collected and have been reported elsewhere.<sup>9</sup> We were able to obtain follow-up data and testing on 130 infants of term control pregnancies and 89 infants of prolonged pregnancies at 1 year of age. There were 111 infants of term control pregnancies and 76 infants of prolonged pregnancies tested at 2 years of age.

The infants were classified according to certain phys-

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**Table I.** Heights and weights at 1 and 2 years

Group	Year 1			Year 2		
	No. of patients	Height (cm)	Weight (kg)	No. of patients	Height (cm)	Weight (kg)
Normal term pregnancy	121	78 ± 4	10.2 ± 1.3	102	89 ± 4	12.9 ± 1.3
Normal prolonged pregnancy	89	77 ± 3	10.4 ± 1.3	76	88 ± 4	13.3 ± 1.9
Prolonged pregnancy, dysmaturity	13	77 ± 3	10.0 ± 0.8	12	87 ± 5	12.4 ± 1.9

**Table II.** General intelligence quotient scores, year 1

Group	No. of patients	General intelligence quotient
Term pregnancies (combined)	130	119 ± 9
Normal	121	119 ± 9
Mild dysmaturity	7	121 ± 6
Advanced dysmaturity	2	121 ± 1
Prolonged pregnancies (combined)	89	119 ± 9
Normal	76	118 ± 8
Mild dysmaturity	7	122 ± 11
Advanced dysmaturity	6	125 ± 10
Combined normal	187	119 ± 9
Combined dysmaturity	22	120 ± 9

**Table III.** General intelligence quotient scores, year 2

Group	No. of patients	General intelligence quotient
Term pregnancies (combined)	111	116 ± 13
Normal	102	116 ± 14
Mild dysmaturity	7	123 ± 11
Advanced dysmaturity	2	122 ± 10
Prolonged pregnancies (combined)	76	116 ± 15
Normal	64	116 ± 15
Mild dysmaturity	6	112 ± 13
Advanced dysmaturity	6	125 ± 19
Combined normal	166	116 ± 14
Combined dysmaturity	21	120 ± 13

ical characteristics at birth into normal or dysmature (mild or moderate dysmaturity). Criteria for dysmaturity were described previously.<sup>9</sup> The following information was obtained during follow-up sessions: (1) height and weight, (2) number of hospital admissions, and (3) developmental assessment scores.

The Griffiths Mental Development Scales were used to assess the infants' development.<sup>10</sup> The Griffiths scales are standardized to 8 years of age, as compared with 2½ years for the Bayley scales.<sup>11</sup> These scales are considered by some to be technically superior to the Gessell test, from which the Bayley, Cattell, Denver, Griffiths, and other tests were derived.<sup>12</sup> The Griffiths scales are based on five subscales in the first year: locomotor, personal-social, hearing and speech, eye and hand coordination, and performance. After the first year a sixth subscale, practical reasoning, is added. Developmental function is therefore split into a broader number of dimensions than, for example, with the Bayley test. A general intelligence quotient score is derived by averaging the subscale scores. All tests were performed by a single, qualified, experienced Griffiths tester (C.-J. C.), in the children's homes. The tester was blinded to the infants' gestational age at delivery.

The scales of Blishen and McRoberts<sup>13</sup> were used to compare socioeconomic distribution between the control and prolonged pregnancy groups. Each family was placed into one of the six classes described by Blishen and McRoberts.

The  $\chi^2$  contingency tables were used to compare the

socioeconomic class distributions. Student's *t* test was used to compare the general intelligence quotient and subscale intelligence quotient scores for infants of term versus prolonged pregnancies and of normal versus dysmature infants. Analysis of variance was used to compare scores for subgroups.

## Results

**Socioeconomic class.** With the use of the Blishen-McRoberts scales, 76% of the term control and 78% of the prolonged pregnancy families were within the upper three socioeconomic classes, and the distributions over the six classes were not significantly different in the first- or second-year follow-up groups.

**Heights and weights.** At 1 year of age the mean heights and weights of infants from normal term control pregnancies, normal prolonged pregnancies, and prolonged pregnancies with dysmaturity are shown in Table I. There were no significant differences for height and weight at 1 and 2 years for each of these subgroups.

**Hospitalizations.** There were only four hospitalizations among all infants studied in the first 2 years. Illnesses included otitis media (two), febrile seizure, and operation for a bony skull abnormality. No significant difference was found for the number of hospitalizations in each group.

**Year 1 developmental testing.** The general intelligence quotient test scores at 1 year of age for the various subgroups are shown in Table II. There were no

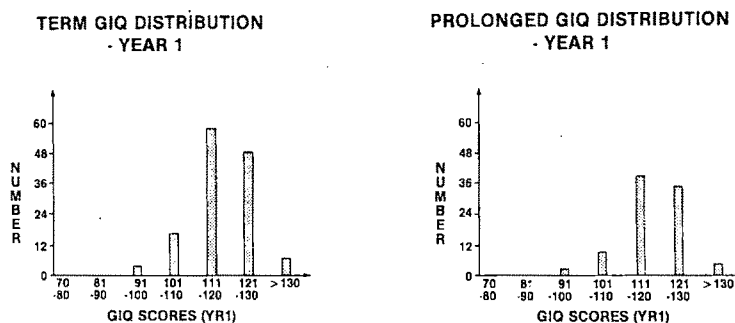


Fig. 1. General intelligence quotient (GIQ) distributions for year 1.

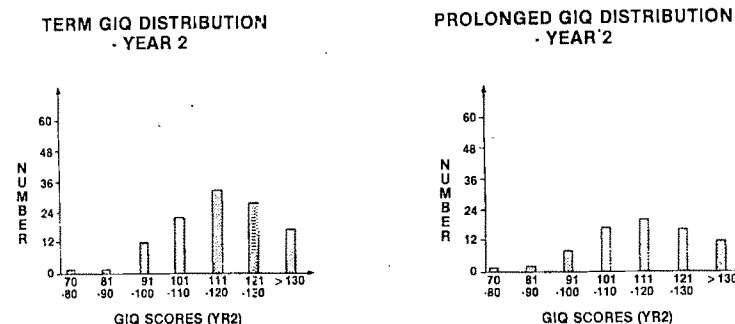


Fig. 2. General intelligence quotient (GIQ) distributions for year 2.

significant differences in general intelligence quotient scores between overall term and prolonged pregnancy infants. No difference was found in general intelligence quotient scores between normal and dysmature infants. Comparison of the various subgroups also showed no significant difference.

**Year 2 developmental testing.** The corresponding results for year 2 are shown in Table III. Similarly, no significant difference was found between the groups after 2 years.

**Developmental score distributions.** The distributions of general intelligence quotient scores of infants of term and prolonged pregnancies are shown for years 1 and 2 in Figs. 1 and 2, respectively. The distributions are similar for each year.

**Subscale comparisons.** Examination of all subscale scores at years 1 and 2 for each subgroup yielded only a single significant difference. Locomotor intelligence quotient subscores in year 2 were significantly greater for the combined group of term pregnancy infants than for combined prolonged pregnancy infants ( $p = 0.025$ ). When normal and dysmature infants from prolonged pregnancies were compared with combined term pregnancy infants, the latter had significantly higher locomotor intelligence quotient scores than infants of normal prolonged pregnancies ( $p = 0.019$ ), but interestingly no difference was found between combined term and dysmature infants of prolonged pregnancies. No difference was found between

normal term infants (not dysmature) and infants of prolonged pregnancies.

**Induced versus spontaneous labor.** Of the prolonged pregnancies that were followed up, labor had been induced in 37%. At 1 year, the infants from term control pregnancies, prolonged pregnancies with spontaneous labor, and prolonged pregnancies with induced labor had mean general intelligence quotient scores of  $119 \pm 9$ ,  $120 \pm 9$ , and  $118 \pm 9$ , respectively. At 2 years, the mean scores were  $116 \pm 13$ ,  $116 \pm 15$ , and  $118 \pm 15$ , respectively. There was no significant difference between the mean scores for these three groups in each year. Furthermore, the evaluation of all subscale scores failed to reveal a single significant difference for either year.

### Comment

Limited scrutiny of early childhood after prolonged pregnancy has suggested disturbances of physical, cognitive, and behavioral development. Zwerdling<sup>5</sup> demonstrated a higher infant mortality up to 2 years of age and an increase in hospitalization rate in the first 3 years. By 5 years of age, however, the 19 postterm infants studied showed no difference in growth or intelligence compared with the accepted mean values for that age.

Field et al.<sup>6</sup> compared 40 postterm and 40 control infants in the first year of life. The postterm, post-mature infants had more perinatal complications, lower



Brazelton and lower motor scores at birth. At 4 months of age, they scored lower on the Denver scale, and at 8 months, while their daily motor scores were similar to those of control infants, their mental scores were lower. They also had more illnesses and feeding and sleep disturbances.

Lovell<sup>7</sup> compared 77 infants beyond 42 weeks and 37 beyond 43 weeks with 106 term control infants. The postterm infants showed more severe illness and sleep defects during the first year and an increased proportion with low social quotients at the 1-year examinations. Babies beyond 43 weeks' gestation were more severely affected. Adverse physical signs during the neonatal period were meconium staining of the cord and nails, subcutaneous wasting, and adducted thumbs. He suggested that the problems of postmaturity could be avoided by induction of labor prior to 42 weeks' gestation.

Callenbach and Hall<sup>8</sup> retrospectively reviewed 53 postterm infants that had been admitted to a neonatal intensive care unit and were subsequently followed up from 6 months to 6 years. They demonstrated that neonatal morbidity and developmental outcome were the same in eutrophic and malnourished infants. They concluded that postterm infants should be considered at risk, even if normally grown. Therefore, while the perinatal morbidity and mortality associated with extended gestation appear to result from placental insufficiency and clinical dysmaturity, the long-term sequelae of prolonged pregnancy may not be limited to the malnourished infant.

Ting et al.<sup>14</sup> compared dysmature and normal control infants at 1, 4, and 7 years in a black population and found no difference in growth, neurological examinations, and psychological testing between the two groups. Their study group included dysmature infants from both term and postterm pregnancies.

In the present study all patients were entered into the study prior to delivery of their infants and followed up in the same perinatal unit by a small group of obstetricians. Only uncomplicated pregnancies with meticulous dating were included for study. The same tester gathered all follow-up data, blinded to the gestational age and condition at birth of the infants. We believe these controlled conditions allowed an accurate and unbiased comparison between the study and control groups.

White and Watts<sup>15</sup> pointed out that important or predictive developmental divergence first becomes clear during the second year of life. From analysis of our data, no significant difference in growth, hospitalization, and overall developmental assessment was found between the prolonged and term pregnancies, and subgroups of each, in the first 2 years of life.

An interesting finding was that of significantly higher locomotor subscale test scores for infants from term control pregnancies as compared with scores of infants from normal prolonged (nondysmature) pregnancies in the second year of testing. Further follow-up testing, which is presently being carried out, will be required to see if this is a significant trend.

In the first part of this study,<sup>9</sup> dysmaturity was found to be a significant perinatal risk factor. In particular, the advanced dysmature group had a significantly greater incidence of low biophysical profile scores, intrapartum fetal distress, emergency cesarean sections, and low Apgar scores ( $p < 0.001$  for each). However, in the first 2 years of follow-up, no developmental differences were found within the limits of our testing methods for prolonged (normal or dysmature) pregnancies versus control pregnancies. We are now in the process of following up these patients up to 5 years of age.

It appears that the acute intrauterine growth retardation that occurs over a relatively short span in the dysmature fetus may not have the same clinical relevance as chronic intrauterine growth retardation in early childhood development.<sup>16</sup>

The results to date are reassuring to the parent of a child from a prolonged gestation, particularly if dysmaturity was present in the perinatal period.

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## Maternal, fetal, and newborn complications associated with newborn intracranial hemorrhage

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Two hundred twenty newborn infants with one or more fetal or newborn complications and 54 newborn infants without fetal or newborn complications were prospectively studied to assess the relationship between maternal, obstetric, fetal, and newborn complications and intracranial hemorrhage. Intracranial hemorrhage occurred in 47 newborn infants with fetal or newborn complications (21%) and in one infant with no fetal or newborn complications (2%). Maternal and obstetric complications, duration of labor, and mode of delivery were not associated with intracranial hemorrhage. Newborn immaturity at delivery is an important factor in the occurrence of intracranial hemorrhage. There is little evidence that fetal hypoxia is a contributing factor. Severe respiratory complications and major infections are newborn complications associated with intracranial hemorrhage. (*AM J OBSTET GYNECOL* 1986;154:345-51.)

**Key words:** Immaturity, hypoxia, intracranial hemorrhage

Computerized tomography and ultrasound examination of the newborn infant provide an accurate diagnosis of newborn intracranial hemorrhage and associated pathologic conditions.<sup>1</sup> These diagnostic techniques enable one to document the natural history of intracranial hemorrhage in the newborn infant and investigate its clinical correlates.

Serial ultrasound examinations in the newborn indicate that the majority of intracranial hemorrhage occurs in the first 3 days of life with 30% to 40% of cases occurring within 24 hours of delivery.<sup>2,3</sup>

Rational prevention programs require an understanding of the pathogenesis of intracranial hemorrhage. A number of studies in very low birth weight infants have reported significant antecedent clinical

events associated with intracranial hemorrhage. However, intracranial hemorrhage also occurs in larger preterm newborn infants and is periodically observed in term newborn infants. Therefore, the present study was designed to examine the association between maternal, fetal, and newborn complications and intracranial hemorrhage in a selected population of infants who spanned a broad range of gestational ages and were at risk because of one or more fetal-newborn complications. Such a population encompasses infants experiencing a wide range of insults and outcomes and provides a sample in which the impact of specific fetal or newborn complications can be factored out from the influence of fetal immaturity per se.

### Methods

The study includes 274 preterm and term patients. There were 220 patients with one or more fetal or newborn complications (index group) and 54 patients with no fetal or newborn complications (control group). The criteria for inclusion in the index group included: (1) very low birth weight (<1500 gm), (2) fetal growth retardation, (3) a diabetic mother, (4) intrapar-

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**Table I.** Numbers of preterm and term newborn infants in index group and in control group of study population

<i>Infants</i>	<i>Index group</i>	<i>Control group</i>
Preterm <1500 gm	70	0
Preterm >1500 gm	79	24
Term	71	30

tum fetal hypoxia, (5) moderate or severe newborn respiratory complications, (6) major newborn infection, and (7) moderate and severe newborn encephalopathy. The control group included both term and preterm newborn infants without fetal or newborn complications born to mothers without maternal or obstetric complications (Table I).

The following maternal characteristics and maternal and obstetric complications were assessed for each obstetric patient and her pregnancy for analysis in this study.

1. Maternal characteristics included age, height, weight, smoking habits, and socioeconomic status.

2. Prior pregnancy wastage included stillbirth and neonatal death in a previous pregnancy.

3. Maternal medical complications included essential hypertension (blood pressure >140/90) confirmed before pregnancy or identified before 20 weeks of gestational age, chronic renal disease, maternal insulin-dependent diabetes, and a miscellaneous group that included acute infections during pregnancy requiring hospitalization, collagen diseases, major neurological or endocrinologic complications, and abdominal operation during pregnancy.

4. Obstetric complications included toxemia, defined by a blood pressure >140/90 mm Hg and/or significant albuminuria, antepartum hemorrhage due to placenta previa or premature placental separation, and a miscellaneous group including severe nausea and vomiting, polyhydramnios, and oligohydramnios.

5. Gestational complications included preterm delivery (<37 weeks), postterm delivery (>42 weeks), multiple pregnancy, clinically suspected fetal growth retardation, clinically suspected anomalies, and clinically suspected isoimmunization.

6. Labor complications included moderate and severe meconium, abnormal uterine action, defined as abnormal contractions with delayed cervical dilatation or labor in excess of 24 hours, radiologically confirmed cephalopelvic disproportion, breech presentation, mid-forceps delivery, and cesarean section.

The following fetal or newborn characteristics and complications were documented for analysis in this study: (1) maturity, measured by gestational age and

**Table II.** Ultrasound classification of intracranial hemorrhage and associated pathologic conditions

<i>Minor</i>	
Grade I. Subependymal hemorrhage	
	Unilateral <10 mm
	Unilateral >10 mm
	Bilateral <10 mm
	Bilateral >10 mm
Grade II. Intraventricular hemorrhage	
	No dilatation
<i>Major</i>	
Grade III. Intraventricular hemorrhage	
	Mild dilatation (4-6 mm)
	Moderate dilatation (7-10 mm)
	Severe dilatation (>10 mm)
Grade IV. Parenchymal hemorrhage, ischemia, infarction	
	Unilateral ≤15 mm
	Unilateral >15 mm
	Bilateral ≤15 mm
	Bilateral >15 mm (one or both)
Grade V. Parenchymal cystic lesions	
	Unilateral ≤15 mm
	Unilateral >15 mm
	Bilateral ≤15 mm
	Bilateral >15 mm

birth weight; (2) fetal growth retardation (excluding major anomalies) in which birth weight was less than the third percentile for gestational age; (3) major anomalies, excluding genetically determined and central nervous system anomalies; (4) infants of diabetic insulin-dependent mothers; (5) fetal hypoxia, biochemically defined by an umbilical arterial buffer base determination <34 mmol/L; (6) newborn respiratory complications, graded as moderate if requiring continuous positive airway pressure and severe if requiring mechanically assisted ventilation; (7) major infection (septicemia or meningitis); (8) newborn hypoglycemia, defined as a blood sugar <1.1 mmol/L in preterm infants and <1.6 mmol/L in term infants; (9) newborn encephalopathy, consisting of clinical neurological signs, defined as moderate in the presence of lethargy and hypotonia and severe in the presence of seizures and/or recurrent apnea.

Ultrasound examinations were performed between day 1 and day 4 after birth, on day 7, at discharge, at 6 months' corrected age, and whenever clinically indicated. A mechanical sector probe (5MHz transducer, Philips Model SDU-3000) was placed on the anterior fontanelle to obtain a series of coronal and sagittal images, which were recorded on transparent film. All observed abnormalities were documented and measured; these included intracranial hemorrhage (subependymal, intraventricular, intraparenchymal, subdural) and associated pathologic conditions such as ventriculomegaly, subependymal cysts, and porencephalic cysts.

A number of grading systems have been proposed

**Table III.** Frequency of intracranial hemorrhage in children with (index group) and without (control group) fetal-newborn complications

Infants	Index group			Control group		
	Total No.	Intracranial hemorrhage		Total No.	Intracranial hemorrhage	
		n	%		n	%
Preterm <1500 gm	70	27	39	0		
Preterm >1500 gm	79	16	20	24	0	0
Term	70	4	6	30	1	3

**Table IV.** Grade of intracranial hemorrhage in preterm and term newborn infants

Intracranial hemorrhage	Preterm		Term (n = 5)	Total
	<1500 gm (n = 27)	>1500 gm (n = 16)		
Minor				
Grade I	12	7	0	19
Grade II	2	0	0	2
Major				
Grade III	4	3	4	11
Grade IV	2	1	0	3
Grade V	7	5	1	13

to classify the range of hemorrhagic and ischemic lesions observed in newborn infants. Abnormal findings in the present study were classified according to the criteria outlined in Table II on the basis of the most severe ultrasound finding observed during the neonatal period or at the 6-month examination.

A preliminary statistical examination of the relationship between maternal characteristics, obstetric complications, and fetal-newborn complications and intracranial hemorrhage was done with the use of a two-way cross-tabulation and  $\chi^2$  analysis. This identified the independent variables appropriate for further analysis. The grade of intracranial hemorrhage has been presented for descriptive purposes. However, for analysis in this study, each newborn infant was classified as either normal or abnormal for intracranial hemorrhage. Then a stepwise logistic regression analysis was used to examine the multivariate associations of the significant independent variables.

## Results

The incidence of intracranial hemorrhage in newborn infants with fetal or newborn complications (index group) in relation to newborn infants with no fetal or newborn complications (control group) is outlined in Table III.

The grade of intracranial hemorrhage in the preterm and term newborn infants is presented in Table IV.

**Table V.** Significance of relationship of obstetric and newborn complications

Complication	Maturity	Respiratory complications
Premature ruptured membranes	p < 0.0001	p < 0.0001
Antepartum hemorrhage	p < 0.0001	p < 0.0001
Isoxsuprine	p < 0.0001	p < 0.0001

Obstetric complications, antepartum hemorrhage, premature rupture of the membranes, and isoxsuprine therapy demonstrated a significant relationship to fetal-newborn immaturity and severe newborn respiratory complications.

**Table VI.** Relationship of duration of labor or method of delivery and intracranial hemorrhage

Method of delivery	Total No.	Intracranial hemorrhage		p
		n	%	
Elective cesarean section, no labor	14	2	14	
Vaginal delivery				
Labor <6 hr	49	9	18	NS
Labor 6-12 hr	37	7	19	
Labor >12 hr	40	8	20	
Vaginal delivery				
Spontaneous	121	26	21	NS
Low forceps	16	1	6	
Midforceps	19	3	16	
Breech	12	2	17	
Cesarean section, labor	94	16	17	

No significant relationship was demonstrated between the duration of labor or the method of delivery and intracranial hemorrhage.

The intracranial hemorrhage was minor in 21 (50%) of the preterm newborn infants. The minor lesions included eight cases of unilateral and 10 cases of bilateral subependymal hemorrhage and two cases of intraventricular hemorrhage without ventricular dilatation. The major lesions included seven instances of intraventricular hemorrhage with ventriculomegaly, three instances of parenchymal hemorrhage, and 12



**Table VII.** Apgar scores in infants with intracranial hemorrhage

Type of delivery	Apgar score	Intracranial hemorrhage				
		Normal		Abnormal		p
		n	Mean	n	Mean	
Vaginal delivery	1 min	136	6.8	30	5.4	0.007
	5 min	104	8.4	24	7.5	0.02
Cesarean section	1 min	78	4.9	16	4.6	NS
	5 min	66	7.7	16	7.9	NS

Newborn infants with intracranial hemorrhage had significantly lower Apgar scores after vaginal delivery.

**Table VIII.** Relationship between fetal metabolic acidosis at delivery and intracranial hemorrhage

Umbilical arterial buffer base (mmol/L)	Total No.	Intracranial hemorrhage		p
		n	%	
>36	156	19	12	NS
34-36	19	2	11	NS
<34	55	9	17	NS

There was no relationship between fetal metabolic acidosis at delivery and intracranial hemorrhage.

instances of porencephalic cysts with or without hemorrhage. Although intracranial hemorrhage was uncommon in the term newborn infant, when found, it was major, consisting of four cases of ventriculomegaly and one case of porencephalic cyst.

The relationship between maternal characteristics, maternal medical complications, obstetric complications, and intracranial hemorrhage was tested by  $\chi^2$  analysis. Three variables, antepartum hemorrhage ( $p < 0.0001$ ), premature rupture of the membranes ( $p < 0.0001$ ), and isoxsuprine therapy for preterm labor ( $p < 0.001$ ), demonstrated a significant association with intracranial hemorrhage in this initial analysis. Because of the association of these variables with the significant fetal or newborn complications, none of these variables showed independent significance with intracranial hemorrhage in subsequent regression analysis (Table V).

Table VI indicates that the incidence of intracranial hemorrhage is not influenced by the duration of labor. Similarly the incidence of intracranial hemorrhage was of the same order in newborn infants delivered vaginally and in those delivered by cesarean section.

The newborn infants delivered vaginally who exhibited lower Apgar scores at 1 and 5 minutes did demonstrate an association with intracranial hemorrhage (Table VII). A number of factors can contribute to low Apgar scores. In the present study, there was no relationship between the duration of labor and Apgar

scores. There was a significant correlation between umbilical arterial buffer base and Apgar scores at 1 minute (R.40) and at 5 minutes (R.48). However, Table VIII demonstrates that the incidence of intracranial hemorrhage does not increase in newborn infants with severe metabolic acidosis (umbilical arterial buffer base  $< 34$  mmol/L) at delivery.

Serial periodic blood gas and acid-base data during the neonatal period were available for some but not all preterm newborn infants. There was no significant difference for mean oxygen tensions in newborn infants with and without intracranial hemorrhage during the 3 days after delivery. Table IX indicates that there was a significantly lower buffer base during the three days after delivery in preterm newborn infants with an intracranial hemorrhage.

The fetal-newborn complications with a significant association with intracranial hemorrhage on initial  $\chi^2$  analysis include fetal-newborn immaturity ( $p < 0.0001$ ), patent ductus arteriosus ( $p < 0.0001$ ), newborn infection ( $p < 0.0001$ ), newborn respiratory complications ( $p < 0.0001$ ), and newborn encephalopathy ( $p < 0.0001$ ). Table X indicates that on subsequent regression analysis, examining the relationship of these fetal or newborn complications with intracranial hemorrhage, the complications with an independent association with intracranial hemorrhage are newborn immaturity, infection, and respiratory complications.

The relationship between newborn infection and intracranial hemorrhage in the three maturity categories are outlined in Table XI. This indicates that major newborn infections are associated with intracranial hemorrhage in the preterm newborn infant  $> 1500$  gm and the term newborn infant.

The relationship between newborn respiratory complications and intracranial hemorrhage in the three maturity categories is outlined in Table XII. This indicates that moderate and severe newborn respiratory complications are associated with intracranial hemorrhage only in the preterm newborn infant  $> 1500$  gm at birth. Pneumothorax occurred as a complication in 18 newborn infants but did not demonstrate a significant association with intracranial hemorrhage.

**Table IX.** Buffer base measurements in preterm newborn infants with and without intracranial hemorrhage during day 1 to day 3 of the newborn period

Day	Buffer base (mmol/L)					
	Preterm infants <1500 gm			Preterm infants >1500 gm		
	Normal	Intracranial hemorrhage	p	Normal	Intracranial hemorrhage	p
Day 1						
0-12 hr	42.3	39.3	0.0001	40.4	39.5	NS
12-24 hr	42.6	40.2	0.0001	40.8	39.5	NS
Day 2	41.5	40.3	0.03	42.0	39.7	0.0001
Day 3	42.5	40.7	0.005	44.5	41.9	0.005

### Comment

The incidence of intracranial hemorrhage of 39% in surviving preterm newborn infants <1500 gm is in keeping with many recent reports.<sup>1, 2, 4-6</sup> However, the incidence of intracranial hemorrhage of 20% in preterm newborn infants >1500 gm and 5% in term newborn infants emphasizes that more mature newborn infants with one or more fetal or newborn complications are also at risk.

The maternal and obstetric complications that are used to define the high-risk pregnancy were analyzed in this study. None demonstrated an independent association with intracranial hemorrhage. Traditional risk scoring of obstetric patients and their pregnancies is not a predictor of intracranial hemorrhage in the newborn infant. However, antepartum hemorrhage and premature rupture of the membranes are predictive of premature labor and newborn immaturity.

Fetal mechanical stress during labor may contribute to intracranial hemorrhage in the immature fetus. This may be due to head compression, which in the fetal lamb has been demonstrated to cause decreased cerebral blood flow.<sup>7</sup> Results to date investigating this question on the basis of the association between the duration of labor and mode of delivery are inconclusive. The absence of an association with either the duration of labor or the mode of delivery in this study is in keeping with results in some reports.<sup>5, 8, 9</sup> However, an association between the duration of labor and intracranial hemorrhage has been observed in a number of studies.<sup>4, 10, 11</sup> Although the incidence of intracranial hemorrhage with cesarean section is consistently lower, no statistically significant advantage of cesarean section after labor over vaginal delivery has been reported.<sup>6, 9, 10</sup> Clarification of the relevance of fetal mechanical stress will require more discriminating measures of the stress of labor and delivery.

The current hypothesis states that intracranial hemorrhage is due to some combination of fetal newborn hypoxia and alterations of systemic blood pressure and cerebral blood flow.<sup>12</sup> Systemic blood pressure may be

**Table X.** Logistic regression analysis of relationship of fetal-newborn complications to intracranial hemorrhage

Complication	$\chi^2$	p
Fetal maturity	9.7	0.002
Infection	9.9	0.002
Respiratory complications	4.0	0.04

of critical importance because, with loss of autoregulation, alterations of systemic pressure will lead to corresponding alterations of cerebral blood flow. Loss of autoregulation has been demonstrated in studies of cerebral blood flow in stressed newborn infants.<sup>13</sup> However, clinical studies have not yet confirmed this hypothesis.

There is little evidence that fetal hypoxia is associated with intracranial hemorrhage. There was no association in this study between intrapartum fetal hypoxia with severe metabolic acidosis at delivery and intracranial hemorrhage. This is in keeping with several recent reports.<sup>10, 14</sup> There was a relationship between low Apgar scores at 1 and 5 minutes and intracranial hemorrhage. The correlation between low umbilical arterial buffer base at delivery and low Apgar scores in preterm and term newborn infants suggests that relative degrees of fetal hypoxia may have a limited association with intracranial hemorrhage.

Immaturity has an independent association with intracranial hemorrhage. This confirms that the degree of newborn maturity at delivery, independent of the complications that occur in these low birth weight infants, is significant. The occurrence of intracranial hemorrhage in the immature fetus and newborn infant is attributed in part to the fragility of the vascular architecture in the germinal matrix, making it vulnerable to changes in cerebral arterial and venous pressure.

Newborn respiratory complications have an independent association with intracranial hemorrhage. This is in keeping with previous reports.<sup>1, 2, 4, 5, 8, 16</sup> However this relationship was not present in the preterm infant

**Table XI.** Association between newborn infection and intracranial hemorrhage in the three maturity categories

Infants	Infection	Total No.	Intracranial hemorrhage		p
			n	%	
Term	None-minor	98	3	3	0.0001
	Major	4	2	50	
Preterm >1500 gm	None-minor	97	13	13	0.001
	Major	4	3	75	
Preterm <1500 gm	None-minor	60	23	38	NS
	Major	10	4	40	

**Table XII.** Association between newborn respiratory complications and intracranial hemorrhage in the three maturity categories

Infants	Respiratory complications	Total No.	Intracranial hemorrhage		p
			n	%	
Term	None-mild	64	5	5	NS
	Moderate	1	0		
	Severe	5	0		
Preterm >1500 gm	None-mild	61	4	7	0.0001
	Moderate	26	4	15	
	Severe	13	8	62	
Preterm <1500 gm	None-mild	27	12	44	NS
	Moderate	12	5	42	
	Severe	3	10	32	

weighing <1500 gm, where the incidence of intracranial hemorrhage was the same in newborn infants without moderate or severe respiratory complications as in newborn infants with respiratory complications. This further emphasizes the importance of immaturity in the occurrence of this complication.

The association of newborn respiratory complications was limited to the preterm newborn infant >1500 gm. This is in keeping with a previous report of Garcia-Prats et al.<sup>15</sup> This suggests that significant maturation has been achieved in these preterm newborn infants >1500 gm so that an additional stress such as a newborn respiratory complication is required for an intracranial hemorrhage to occur. Hypoxemia is presumed to be a mediator of this relationship. However that linkage has not been established. The results of the relationship of abnormal blood gas measures to intracranial hemorrhage in the literature have been contradictory.<sup>2,4,5,8</sup> There was no relationship between newborn blood gas tensions and intracranial hemorrhage in this study. The association between low newborn buffer base during the 3 days after delivery and intracranial hemorrhage in this study is similar to the results reported by Cooke<sup>16</sup> in 1981. This indicates that some degree of newborn hypoxia has been present during this critical period. These apparent discrepancies in regard to newborn hypoxemia may be due to the discontinuity of the random newborn blood gas data

analyzed in most studies to date. It highlights the need for a continuous blood gas measure to assess this relationship.

There were a small number of major newborn infections such as septicemia and meningitis. However, in spite of the small numbers, this complication demonstrated an independent association with intracranial hemorrhage. This association occurred in preterm newborn infants >1500 gm and term newborn infants. This observation, in conjunction with an association of infection with subsequent motor and cognitive deficits,<sup>17</sup> suggests that major infections are an important mechanism of cerebral injury in a small number of newborn infants.

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## An evaluation of different media for the zona-free hamster egg penetration test

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Hypertonic high-salt Biggers, Whitten, and Whittingham medium was compared with either a complex culture medium (synthetic tubal fluid) or a variant of Tyrode's medium for capacitating donor human spermatozoa to be used in the zona-free hamster egg penetration test. Five sperm preparations showed a slightly higher penetration rate in synthetic tubal fluid than in high-salt Biggers, Whitten, and Whittingham medium, five showed equivalent results, and only two had slightly lower penetration rates. However, in modified Tyrode's medium only one sample showed a lower penetration, while six showed comparable results and five showed higher rates of penetration. This last group included two samples that had demonstrated zero penetration in high-salt Biggers, Whitten, and Whittingham medium. Although no overall significant differences between media in the two series of experiments could be discerned with paired *t* tests, in view of the lower incidence of false negative results in modified Tyrode's medium and the absence of osmotic stress, this medium may represent a better choice for routine performance of the hamster egg penetration test. (*AM J OBSTET GYNECOL* 1986;154:351-4.)

**Key words:** Zona-free hamster egg penetration test, culture media, human spermatozoa

Since the original report by Yanagimachi et al.<sup>1</sup> that capacitated and acrosome-reacted human spermatozoa were able to penetrate zona pellucida-free hamster oo-

cytes, the hamster egg penetration test has received considerable attention both as a diagnostic procedure for male infertility and as a research tool.<sup>2,3</sup>

A major difficulty regarding the use of the hamster egg penetration test as a routine diagnostic procedure is the extreme variability in methodology used in different laboratories.<sup>2,3</sup> This variability is particularly important since it is now known that the maximum proportion of zona-free hamster oocytes that may be penetrated by a given sperm population is dependent upon the duration of the capacitation incubation.<sup>4</sup> This optimum capacitation time is variable between men, and

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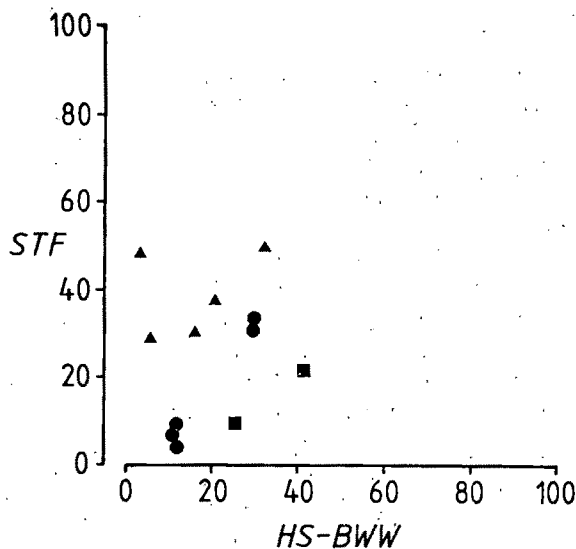


Fig. 1. Scatter plot illustrating the relationship between the percentages of zona-free hamster oocytes penetrated by spermatozoa preincubated in either high-salt BWW medium (HS-BWW) or synthetic tubal fluid (STF) (12 experiments). Circles denote comparable results between the two media; triangles and squares, those that were appreciably higher or lower in synthetic tubal fluid than in high-salt BWW medium.

therefore a standard preincubation time is difficult to select. The alternative solution of repeating the test after different durations of preincubation makes the hamster egg penetration test an even more cumbersome procedure and not really practicable for routine purposes.

One approach to solving this problem is the use of a hypertonic modification of the standard Biggers, Whitten, and Whittingham (BWV) medium,<sup>5</sup> along the lines of the hypertonic pretreatment necessary to capacitate rabbit spermatozoa in vitro.<sup>6</sup> This high-salt BWV medium<sup>7</sup> has the advantages of: (1) reducing the incidence of false negative results in the hamster egg penetration test and (2) enabling the test to be completed within one working day.

The hamster egg penetration test is generally accepted to have inherent deficiencies with regard to the existence of false negative results and uncertain predictive value of positive test results. However, the only thorough prospective study reported to date has shown the test to be of some significant prognostic value in the prediction of male fertility.<sup>8</sup>

Unfortunately, hypertonic treatment is sometimes deleterious to the spermatozoa. This is particularly a problem with the spermatozoa of many oligozoospermic men. In addition many cryopreserved semen samples show poor tolerance of hypertonic stress. In an attempt to find an alternative to the high-salt BWV medium we compared two other media known to be capable of supporting the capacitation of human sper-

matozoa within a few hours in respect to the levels of penetration found in the hamster egg penetration test.

### Material and methods

**Media.** The high-salt BWV medium was prepared according to the original method described by Aitken et al.<sup>7</sup> and typically has an osmolality of 410 mosm. Modified Tyrode's medium<sup>9</sup> and a complex culture medium whose composition is based on human tubal fluid and blood plasma (synthetic tubal fluid<sup>10</sup>) were tested against the high-salt BWV medium. These media are typically of 310 and 285 mosm, respectively.

**Sperm preparation.** Semen samples were obtained from a panel of research donors after requested 3-day periods of prior sexual abstinence. Aliquots of liquefied semen were taken at 30 minutes after ejaculation and spermatozoa were prepared for the capacitation incubation by means of a discontinuous Percoll gradient procedure.<sup>9</sup> The final sperm preparations, which were of 90.3% ( $\pm 7.5\%$  SD;  $n = 19$ ) motility, were washed into the appropriate medium for capacitation and their concentration was adjusted to  $5 \times 10^6$  motile spermatozoa per milliliter.

**Capacitation.** Good hamster egg penetration was known to occur following a 3-hour preincubation in synthetic tubal fluid<sup>10</sup>; therefore, in the synthetic tubal fluid versus high-salt BWV medium comparison this period of preincubation in synthetic tubal fluid was performed under an air atmosphere. The standard conditions of use for the modified Tyrode's medium at the time of this study were 5 hours of preincubation and an atmosphere of 5% carbon dioxide in air. Sperm preincubation in high-salt BWV medium was for 3 hours under 5% carbon dioxide in air. A total of 19 experiments were performed, including 12 comparisons each of synthetic tubal fluid or modified Tyrode's medium versus high-salt BWV medium.

**Oocyte preparation.** Mature female golden hamsters (Charles River) were induced to superovulate by intraperitoneal injections of 40 IU of pregnant mare's serum gonadotropin (Folligon, Intervet, Cambridge, United Kingdom) and 30 IU of human chorionic gonadotropin (Sigma Chemical Co., St. Louis, Missouri) at 70 and 16 hours before killing, respectively. The hamsters were killed by carbon dioxide asphyxiation and cervical dislocation, and the oviducts were excised. Cumulus masses were released from the swollen ampullae and dispersed with the use of 0.1% (w/v) hyaluronidase (Type I-S, Sigma Chemical Co.) in the appropriate medium (standard BWV, synthetic tubal fluid, or modified Tyrode's medium). The isolated oocytes were then transferred through 0.1% (w/v) trypsin (Type I, Sigma Chemical Co.) in protein-free medium followed by three washes in fresh complete medium. For the modified Tyrode's medium and synthetic tubal fluid these

final washes were in media containing the usual 30 or 12 mg/ml of human serum albumin (Cohn Fraction V, Sigma Chemical Co.). However, a high-albumin BWB variant (30 mg of human serum albumin per milliliter as opposed to the usual 3 mg/ml) was used for zona-free oocyte washing and incubation with the spermatozoa.

**Penetration incubation.** After the preincubation period the sperm preparations were readjusted to  $5 \times 10^6$  motile cells per milliliter, and duplicate 20  $\mu$ l droplets were dispensed into 50 mm Falcon tissue culture dishes. Warm liquid paraffin (BDH Chemicals, Toronto, Ontario, Canada) was then poured over the droplets and 20 to 30 zona-free oocytes were transferred to each droplet. Dishes were incubated in anaerobic jars at 37°C. The synthetic tubal fluid dishes were incubated under an air atmosphere, while the BWB and modified Tyrode's medium dishes were incubated under 5% carbon dioxide in air. The durations of the penetration incubations were 3 hours for the synthetic tubal fluid and BWB dishes and 2 hours for the modified Tyrode's medium dishes.

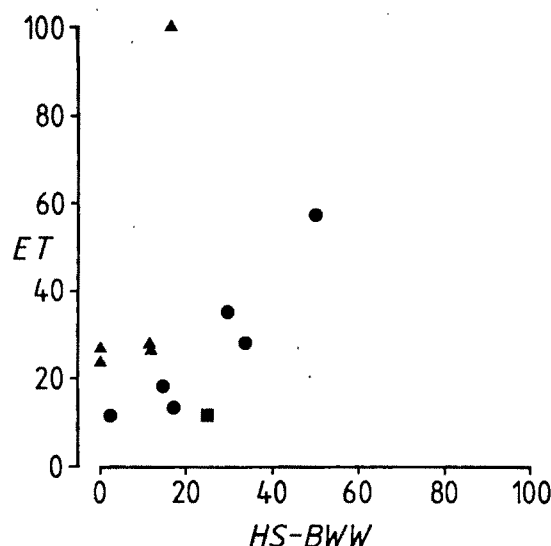
**Scoring.** After the appropriate penetration incubation period the oocytes were washed three times in their respective media and scored under phase contrast optics at magnifications of 400 $\times$  to 500 $\times$  for the presence of swollen sperm heads. Results were expressed as: (1) the penetration rate (percentage of oocytes containing  $\geq 1$  swollen sperm head), (2) the degree of polyspermy (average number of swollen sperm heads per penetrated oocyte), and (3) the penetration capacity (the product of the penetration rate and the degree of polyspermy).

**Statistical analysis.** Differences between media in the two series of comparison experiments were determined by paired *t* tests.

## Results

**Synthetic tubal fluid versus high-salt BWB medium.** The penetration rates for these experiments are shown in Fig. 1. Five sperm preparations showed a slightly higher penetration rate after capacitation in the synthetic tubal fluid than in the high-salt BWB medium, another five showed equivalent penetration rates, and only two showed slightly lower penetration in the synthetic tubal fluid medium. There was, however, no significant difference between the results obtained in the two media ( $t = -1.131$ , 11 df,  $p = 0.282$ ). Polyspermy rates were essentially comparable in the two media (range = 1.0 to 1.8), and when the penetration capacities were calculated, the difference between the two sets of tests remained not significant ( $t = -0.497$ , 11 df,  $p = 0.623$ ).

**Modified Tyrode's medium versus high-salt BWB medium.** The penetration rates for these experiments



**Fig. 2.** Scatter plot illustrating the relationship between the percentages of zona-free hamster oocytes penetrated by spermatozoa preincubated in either high-salt BWB medium (HS-BWB) or modified Tyrode's medium (ET) (12 experiments). Circles denote comparable results between the two media; triangles and squares, those that were appreciably higher or lower in modified Tyrode's medium than in high-salt BWB medium.

are shown in Fig. 2. Only one sample showed a lower rate of penetration after capacitation in modified Tyrode's medium than was found after capacitation in high-salt BWB medium, while six showed equivalent results and five showed higher rates of penetration. Moreover, this last group included two samples that had shown zero penetration after preincubation in high-salt BWB medium (23.3% and 26.7% penetrated oocytes in modified Tyrode's medium) and one that showed only 16.1% (1.1 polyspermy) in high-salt BWB medium but 100% (7.3 polyspermy) in modified Tyrode's medium. In general terms the polyspermy values were equivalent between the two groups, with the exception of the one sample that was scored as 7.3 polyspermy after preincubation in modified Tyrode's medium. The two series of tests were not significantly different with respect to either the penetration rates ( $t = -1.947$ , 11 df,  $p = 0.075$ ) or the penetration capacities ( $t = -1.157$ , 11 df,  $p = 0.271$ ).

## Comment

The experiments described in this article provide clear evidence that media other than high-salt BWB medium are able to support the capacitation of human spermatozoa in short incubation periods. Synthetic tubal fluid, which was developed as a medium for physiologic studies on human sperm function,<sup>10</sup> showed no great advantage over high-salt BWB medium in terms of hamster egg penetration test success, although it

does obviate the need for stressful hypertonic treatment of the spermatozoa. However, the modified Tyrode's medium showed a clear advantage over high-salt BWB medium not only in that the penetration rates and penetration capacities were equivalent to or greater than those in high-salt BWB medium in all but one test but in that there was a reduced incidence of false negative results (two cases). Since high-salt BWB medium is itself considered to result in fewer false negative hamster egg penetration test results, the increased ability of modified Tyrode's medium in this respect, in conjunction with its isotonic formulation, suggests that it may be a better choice for this purpose.

In a later series of experiments it was found that a 3-hour penetration incubation further increased the extent and degree of penetration of the zona-free hamster oocytes.<sup>9</sup> Therefore, a 5-hour preincubation plus a 3-hour penetration incubation format has now replaced the earlier version, which allowed only 2 hours of sperm-oocyte contact. If 45 minutes each is allowed for the production/liquefaction of the semen sample and for sperm preparation by the discontinuous Percoll gradient technique, this format of hamster egg penetration test incorporating the modified Tyrode's medium requires less than 10 hours from arrival of the patient to the time that the eggs are ready for scoring.

This experimental procedure therefore has great potential in the routine application of the hamster egg penetration test as a diagnostic procedure.

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# Microsurgical reversal of sterilization: A six-year study

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A prospective study of 113 personal consecutive microsurgical reversals of female sterilization during the 6-year period from 1979 to 1984 was carried out to determine factors affecting the pregnancy rate. The sterilizations were performed by laparoscopic unipolar coagulation in 54% of the patients, by the Pomeroy technique in 28%, by fimbriectomy in 8%, by the Irving operation in 5%, and by clips or rings in 4%. In the group with no minimum follow-up period, 50% had intrauterine pregnancies and 5% had ectopic gestations. Eighty-nine patients had at least 12 months of follow-up after reversal surgery. This group is studied in detail. Factors affecting the pregnancy rate were length of tube, type of sterilization performed, anastomotic site, and availability of both tubes for reconstruction. Age, parity, and interval from sterilization to reversal surgery did not affect the pregnancy rate. Fifty percent of the intrauterine pregnancies were conceived within 6 months of reversal surgery. (*Am J Obstet Gynecol* 1986;154:355-61.)

**Key words:** Microsurgery, sterilization, reversal of sterilization, ectopic pregnancy

Tubal sterilization has become the most frequently performed intra-abdominal procedure in women of reproductive age in North America.<sup>1</sup> Sterilization is performed in more than 800,000 women annually on this continent and is the most common contraceptive method for women over the age of 30.<sup>2</sup> It has been estimated that of the more than six million women who have been sterilized, up to 10% subsequently regret their decision.<sup>3</sup>

The dramatic rise in separation and divorce rates, along with the publicity in the media regarding increasing success with microsurgical techniques, has increased patient demand for reversal of sterilization operations. It is estimated that 1% of women will seek a reversal of their sterilization.<sup>4</sup>

This study was initiated in 1979 to evaluate the results of a personal consecutive series of reversal of sterilization operations with use of a microsurgical technique and to determine which factors affected the pregnancy rate.

## Subjects and methods

During the 6-year period from January, 1979, to December, 1984, one of us (M. M. S.) operated on 113 consecutive female patients to reverse a previous sterilization. The patients ranged in age from 20 to 43 years, with an average of 31.9 years. The average parity was 2.1, and 10 patients had never been pregnant be-

fore their sterilization. Sixty-six patients (58%) lived within a range of 35 miles from the city of Toronto, while 47 (42%) lived beyond this distance, with many residing several hundred miles from Toronto.

The methods of sterilization are noted in Table I. Unipolar coagulation via the laparoscope accounted for the majority (54%) of cases.

The reasons for requesting reversal are similar to those in Gomel's series.<sup>5</sup> A change in marital status was the principal reason given by 75 (66%) of the patients. Thirty-four (30%) were in the same marital relationship but regretted their original decision. Four patients (4%) requested reversal because of the death of a child.

The interval in months from the original sterilization to the reversal procedure is presented in Table II. Nearly 60% of the sterilizations were carried out more than 5 years before the request for reversal.

A copy of the original sterilization operative report was available and of definite value in only 39 (35%) patients. A report could not be obtained for 24 (21%) of the patients. Fifty (44%) of the operative reports were either inaccurate or too generalized to be of significant value.<sup>6</sup>

A preoperative investigation was performed to determine whether other absolute infertility factors in either partner existed and whether it was feasible to carry out an anastomosis. A normal sperm analysis or a satisfactory postcoital test was required in all cases. Evidence of presumptive ovulation was obtained on all patients by one or more of the following: (1) basal body temperature graphs, (2) elevated progesterone levels in the luteal phase, and (3) endometrial biopsies. Hysterosalpingography was performed on 51 (45%) of the patients to assess the uterine cavity and the intramural and proximal segments of the fallopian tube. A lapa-

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**Table I.** Methods of sterilization

Sterilization method	No minimal follow-up		12 months of follow-up	
	No.	%	No.	%
Coagulation	61	53.9	46	51.6
Pomeroy	32	28.3	27	30.3
Ring/clip	5	4.4	5	5.6
Fimbriectomy	9	7.9	6	6.7
Irving	6	5.3	5	5.6
Total	113		89	

roscopic examination was performed on 100 (85%) of the patients to evaluate the status of the remaining portion of tube and to identify any coexisting pathologic conditions. The reversal surgery was never carried out immediately after the laparoscopic examination. Reversal surgery was scheduled in the early proliferative phase of the cycle, following a final interview with the couple, at which time the prognosis and all details of the proposed surgery were discussed.

The operative technique used in all patients was a meticulous microsurgical procedure similar to the methods reported by Gomel<sup>7</sup> and Winston.<sup>8</sup> A Zeiss OPMI-7 microscope with a 300 mm lens was used for these procedures. A No. 8 pediatric Foley catheter was placed in the uterine cavity to allow intraoperative chromoperturbation throughout the operation.

Prophylactic antibiotics were used before the initial incision and continued for the first 48 hours of the postoperative period. Ringer's lactate solution with 1 gm of hydrocortisone sodium succinate and 3000 U of heparin per liter was used as the irrigating solution.

The anastomoses were carried out in two layers, with use of an 8-0 Vicryl suture; 6-0 Vicryl was used for the mesosalpinx or myometrial closure. A submucosal suture placement technique was used in all cases. Patency was tested, but a water-tight closure was not obtained or insisted on in all cases.

In 22 cases a difficult cornual anastomosis was encountered. For these, a 2-0 nylon suture was left in as a stent for period ranging from 72 to 96 hours. This suture was subsequently removed from the uterine cavity via the vagina with a Burnberg hook. The reimplantation technique used was that described by Peterson et al.<sup>9</sup> A fine silicone rubber tubing was used as a splint and removed within 96 hours following surgery. A dilute Pitressin solution was injected into the myometrium in the cornual anastomoses and the reimplantation procedures.

An omentectomy was carried out in cases with significant pelvic adhesions. An Olshausen type of uterine suspension was performed when there was marked uterine retroversion.

**Table II.** Interval from sterilization to surgery

No. of months from sterilization to surgery	No minimal follow-up		12 months of follow-up	
	No.	%	No.	%
< 12	5	4.4	4	3.5
12 to 23	9	7.9	9	7.9
24 to 35	9	7.9	8	7.0
36 to 59	23	20.3	19	16.8
>60	67	59.2	49	43.3
Total	113		89	

One hundred milliliters of 32% dextran 70, along with 80 mg of methyl prednisolone sodium succinate, was placed into the peritoneal cavity after thorough lavage with Ringer's lactate solution. During the first 48 hours of the postoperative period the following additional adjuvants were used; prophylactic antibiotics, hydrocortisone (100 mg three times daily intravenously), and Phenergan (50 mg four times daily for 48 hours).

In some cases the sterilization was asymmetrical, and consequently different anastomoses were carried out on each tube. The anastomotic site was classified according to the site of anastomosis in the longer tube.

$\chi^2$  contingency tables were used for statistical analysis.

## Results

Of the 113 consecutive patients who underwent a reversal of a previous sterilization, 57 (50%) had one or more subsequent intrauterine pregnancies. There were six (5%) ectopic gestations. Many of these patients had just been seen at their initial postoperative visit, and many had not yet attempted a pregnancy. There was no postoperative morbidity in any patient.

The first consecutive 89 patients had the potential for at least 1 year of follow-up after reversal surgery, and this group has been studied in detail. Of these 89 patients, 49 (55%) had one or more subsequent intrauterine pregnancies. Term deliveries occurred in 40 (45%) and abortions in nine (10%) of the patients. There were six (7%) ectopic pregnancies.

Two patients did not return for a postoperative assessment: an additional 10 patients were seen at a 6-week postoperative visit and then lost to follow-up. All of these patients lived in remote areas and could not be contacted in spite of many attempts to do so. In addition, two patients had a hysterectomy in another center within 12 months of reversal surgery, without prior discussion or consultation with the authors. These 14 patients have been included in the "failure to conceive" group.

The intrauterine pregnancy rate was not affected by the parity of the patient. The pregnancy rate was 61%

**Table III.** Timing of sterilization and outcome

Timing	Total patients	Term pregnancies		Abortions		Intrauterine pregnancies		Ectopic pregnancies*	
		No.	%	No.	%	No.	%	No.	%
Interval	70	32	45.7	7	10.0	39	55.7	2	2.8
Postpartum	19	8	42.1	2	10.5	10	52.6	4	21.0
Total	89	40		9		49		6	

\*p < 0.01.

**Table IV.** Type of anastomosis and outcome

Type of anastomosis	Total patients	Outcome							
		Term pregnancies		Abortions		Intrauterine pregnancies		Ectopic pregnancies	
		No.	%	No.	%	No.	%	No.	%
Isthmus-isthmus	8	7	87.5	0	0.0	7	87.5	0	0.0
Isthmus-cornual	4	3	75.0	0	0.0	3	75.0	0	0.0
Ampullary-isthmus	32	12	37.5	4	12.5	16	50.0	4	12.5
Ampullary-cornual	22	10	45.4	1	4.5	11	50.0	2	9.0
Ampullary-ampullary	2	2	100.0	0	0.0	2	100.0	0	0.0
Reimplantation	15	5	33.3	4	26.6	9	60.0	0	0.0
Salpingostomy	6	1	16.6	0	0.0	1	16.6	0	0.0
Total	89	40		9		49		6	

**Table V.** Sterilization method and outcome

Sterilization method	Total patients	Term pregnancies		Abortions		Intrauterine pregnancies		Ectopic pregnancies	
		No.	%	No.	%	No.	%	No.	%
Coagulation	46	22	47.8	6	13.0	28	60.8	1	2.1
Pomeroy	27	11	40.7	3	11.1	14	51.8	3	11.1
Ring/clip	5	4	80.0	0	0.0	4	80.0	1	20.0
Fimbriectomy	6	1	16.6	0	0.0	1	16.6	0	0.0
Irving	5	2	40.0	0	0.0	2	40.0	1	20.0
Total	89	40		9		49		6	

in the group living within 35 miles of the city, and 47% in the more remote group. All the patients lost to follow-up were in this latter group.

Postpartum sterilizations had been performed on 19 patients (21%), and 70 (79%) had interval sterilizations (Table III). Note that ectopic pregnancies occurred in four of 19 (21%) of the patients who had previous postpartum sterilizations, significantly more than the two of 70 (2.8%) that occurred after interval sterilization ( $p < 0.01$ ).

Four of the six ectopic pregnancies occurred after ampullary-isthmic anastomoses, and two occurred in ampullary-cornual anastomoses (Table IV). In these instances there is a marked disparity in the caliber of the lumen at the anastomotic site.

Of the four ectopic gestations that resulted following

reversal of a previous postpartum sterilization, three had the Pomeroy technique and one had the Irving operation (Table V). Of the two ectopic gestations that developed in the interval sterilization group, one occurred in an ampullary-cornual anastomosis following a previous unipolar coagulation and one occurred in an ampullary-isthmic anastomosis following a Bleier clip application (Table V). The later ectopic gestation occurred in an ampullary diverticulum noted previously at reversal and not at the anastomotic site.

Although the numbers are small, Table IV indicates that the best results were achieved in cases in which there was little or no disparity in the caliber of the lumen of the tubal segments. The isthmic-isthmic and ampullary-ampullary anastomoses produced the highest intrauterine pregnancy results. The poorest results

**Table VI.** Type of anastomosis and outcome

Type of anastomosis	Total patients	Term pregnancies		Abortions		Intrauterine pregnancies*		Ectopic pregnancies	
		No.	%	No.	%	No.	%	No.	%
Unilateral	16	4	25.0	0	0.0	4	25.0	2	12.5
Bilateral	73	36	49.3	9	12.3	45	61.6	4	5.4
Total	89	40		9		49		6	

\* $p < 0.005$ .**Table VII.** Tubal length and outcome

Tubal length	Total patients	Term pregnancies		Abortions		Intrauterine pregnancies*		Ectopic pregnancies	
		No.	%	No.	%	No.	%	No.	%
<4	5	1	20.0	0	0.0	1	20.0	0	0.0
4-6	73	31	42.4	9	12.3	40	54.7	5	6.8
>6	11	8	72.7	0	0.0	8	72.7	1	9.0
Total	89	40		9		49		6	

\* $p < 0.005$ .**Table VIII.** Interval from sterilization to surgery and outcome

No. of months from sterilization to surgery	Total patients	Term pregnancies		Abortions		Intrauterine pregnancies*		Ectopic pregnancies	
		No.	%	No.	%	No.	%	No.	%
<12	4	1	25.0	0	0.0	1	25.0	1	25.0
12-23	9	5	55.5	0	0.0	5	55.5	0	0.0
24-35	8	5	62.5	0	0.0	5	62.5	0	0.0
36-59	19	6	31.5	2	10.5	8	42.1	2	10.5
>60	49	23	46.9	7	14.2	30	61.2	3	6.1
Total	89	40		9		49		6	

\*NS.

were found in the fimbriectomy group, with only one of six women (17%) achieving an intrauterine pregnancy.

The sterilizations performed with application of rings or clips had the highest intrauterine pregnancy rate following reversal surgery (Table V).

A significant difference was found in the intrauterine pregnancy rate if both tubes were available for reconstruction ( $p < 0.005$ ) (Table VI). In unilateral anastomoses, often coexisting pathologic conditions were present, or a more destructive type of sterilization had been performed originally.

Table VII indicates that there is a significant relationship between the total length of tube remaining following reversal surgery and the subsequent intrauterine pregnancy rate ( $p < 0.05$ ). With <4 cm of tube, the rate was 20%, increasing to 55% with 4 to 6 cm of tube and reaching 73% with >6 cm of tube remaining following reconstruction. We could not find a signifi-

cant difference relating length of tube and the interval from surgery to conception time in this series (data not shown).

Table VIII depicts the outcome related to the interval from sterilization to reversal surgery. In 49 patients (43%); this interval exceeded 5 years. This group had an intrauterine pregnancy rate of 61%. The pregnancy rates were not significantly affected by the duration from sterilization to surgery.

No significant difference was found in the outcome related to the use of splints (Table IX).

Forty-six of the 89 patients (52%) had unipolar tubal coagulation with the laparoscope as the sterilization procedure (Table X). In this group, there were 28 (60%) intrauterine pregnancies, 22 (47%) live births, and six (13%) abortions. There was only one ectopic pregnancy (2%) in this group.

Table XI shows the anastomotic sites and outcome for the Pomeroy sterilization group.

**Table IX.** Use of splints and outcome

<i>Splints</i>	<i>Total patients</i>	<i>Term pregnancies</i>		<i>Abortions</i>		<i>Intrauterine pregnancies*</i>		<i>Ectopic pregnancies</i>	
		<i>No.</i>	<i>%</i>	<i>No.</i>	<i>%</i>	<i>No.</i>	<i>%</i>	<i>No.</i>	<i>%</i>
Used	37	14	37.8	6	16.2	20	54.0	2	5.4
Not used	52	25	50.0	3	5.7	29	55.7	4	7.6
Total	89	40		9		49		6	

\*NS.

**Table X.** Anastomosis type and outcome for coagulation sterilization

<i>Type of anastomosis</i>	<i>Total patients</i>	<i>Term pregnancies</i>		<i>Abortions</i>		<i>Intrauterine pregnancies</i>		<i>Ectopic pregnancies</i>	
		<i>No.</i>	<i>%</i>	<i>No.</i>	<i>%</i>	<i>No.</i>	<i>%</i>	<i>No.</i>	<i>%</i>
Isthmus-isthmus	1	1	100.0	0	0.0	1	100.0	0	0.0
Isthmus-cornual	4	3	75.0	0	0.0	3	75.0	0	0.0
Ampullary-isthmus	6	3	50.0	1	16.6	4	66.6	0	0.0
Ampullary-cornual	20	10	50.0	1	5.0	11	55.0	1	5.0
Ampullary-ampullary	0	0	0.0	0	0.0	0	0.0	0	0.0
Reimplant	15	5	33.3	4	26.6	9	60.0	0	0.0
Salpingostomy	0	0	0.0	0	0.0	0	0.0	0	0.0
Total	46	22		6		28		1	

**Table XI.** Type of anastomosis and outcome for Pomeroy sterilizations

<i>Type of anastomosis</i>	<i>Total patients</i>	<i>Term pregnancies</i>		<i>Abortions</i>		<i>Intrauterine pregnancies</i>		<i>Ectopic pregnancies</i>	
		<i>No.</i>	<i>%</i>	<i>No.</i>	<i>%</i>	<i>No.</i>	<i>%</i>	<i>No.</i>	<i>%</i>
Isthmus-isthmus	4	3	75.0	0	0.0	3	75.0	0	0.0
Isthmus-cornual	0	0	0.0	0	0.0	0	0.0	0	0.0
Ampullary-isthmus	20	6	30.0	3	15.0	9	45.0	2	10.0
Ampullary-cornual	1	0	0.0	0	0.0	0	0.0	1	100.0
Ampullary-ampullary	2	2	100.0	0	0.0	2	100.0	0	0.0
Reimplant	0	0	0.0	0	0.0	0	0.0	0	0.0
Salpingostomy	0	0	0.0	0	0.0	0	0.0	0	0.0
Total	27	11		3		14		3	

Table XII indicates that 25 (50%) of the intrauterine pregnancies were conceived within 6 months of reversal surgery. Only three (3%) patients conceived more than 2 years from the reversal operation; one was the only fimbriectomy patient to conceive, and the other two occurred following reimplantation procedures.

Thirty-two of the patients who did not conceive had subsequent hysterosalpingography. In 29 (91%) of these patients, at least one tube was found to be patent. Nine of the patients who did not conceive had laparoscopic examinations. Two patients had significant pelvic adhesions, and five had bilateral patency with no adhesions.

# **Comment**

The 12 patients lost to follow-up within 6 months of reversal surgery are included in the "failure to con-

ceive" group. Some authors exclude patients not followed for at least 6 months in their series.<sup>10</sup> We decided to report our series in this fashion and included all patients, since this procedure depicts a realistic scenario of this complex group of patients seeking reversal. It was perplexing to discover that two patients had a hysterectomy within 1 year of reversal surgery without prior notification or consultation with us. In spite of determined efforts to follow this group of patients, changes of name and address, along with the fact that many patients resided a great distance from the city, made it a difficult task. Although all patients promised to notify us immediately if they thought they were pregnant, in many instances we discovered only by chance through follow-up letters that they had given birth to a child.

Seigler and Perez<sup>11</sup> reviewed the world's literature on



**Table XII.** Type of anastomosis and interval from surgery to conception

Type of anastomosis	Total patients	Intrauterine pregnancies		Months from surgery to conception in intrauterine pregnancies							
		No.	%	<6		6-11		12-23		24-36	
				No.	%	No.	%	No.	%	No.	%
Isthmus-isthmus	8	7	87.5	2	25.0	3	37.5	2	25.0	0	0.0
Isthmus-cornual	4	3	75.0	2	50.0	1	25.0	0	0.0	0	0.0
Ampullary-isthmus	32	16	50.0	10	31.2	1	3.1	5	15.6	0	0.0
Ampullary-cornual	22	11	50.0	5	22.7	3	13.6	3	13.6	0	0.0
Ampullary-ampullary	2	2	100.0	2	100.0	0	0.0	0	0.0	0	0.0
Reimplant	15	9	60.0	4	26.6	1	6.6	2	13.3	2	13.3
Salpingostomy	6	1	16.6	0	0.0	0	0.0	0	0.0	1	16.6
Total	89	49		25		9		12		3	

sterilization reversal in 1975. They reported an overall intrauterine pregnancy rate of only 21% following reversal surgery. More recently there have been reports of pregnancy rates ranging from 50% to 80%.<sup>7, 8, 10</sup> In the 89 patients in our series who had the potential for at least 1 year of follow-up, there were 49 (55%) intrauterine pregnancies, of which 40 (45%) were term deliveries and 9 (10%) were spontaneous abortions. There were six (7%) ectopic pregnancies. The use of magnification, along with a meticulous microsurgical technique, has likely been the most important factor in improving the success of this procedure.

There was no morbidity in any of the 113 patients operated on to reverse their sterilization. There were no serious reactions to any of the prophylactic antibiotics that were used. It has been suggested that the use of steroids would result in faulty healing at the tubal reanastomosis site and also possibly at the abdominal incision. We were unable to find evidence of faulty healing in any case.

Loss of patency was not a significant factor in the group that failed to conceive, since 26 of the 29 patients who had a postoperative hysterosalpingogram revealed at least one patent fallopian tube, and five of the nine laparoscopic examinations in this failure-to-conceive group were perfectly normal. Other factors, such as an inadequate luteal phase, were found in some patients, and they did achieve a pregnancy with clomiphene therapy.

Silber and Cohen<sup>12</sup> found that tubal length was the important factor in their success with reversal of sterilization operations. In this series we have confirmed those observations, by finding a significantly higher success rate after reversal of tubes of >6 cm in length. The results were not improved by a longer follow-up period, as was reported by Gomel.<sup>13</sup>

Vasquez et al.<sup>14</sup> suggested that the results of reversal surgery are much better if the interval from sterilization is <5 years. We could not corroborate this finding in our series, since 30 (61%) of the 49 patients who had

their sterilization >5 years before the reversal surgery had a subsequent intrauterine pregnancy.

Like Winston,<sup>8</sup> we found that the site of anastomosis significantly affected the eventual outcome. An 88% intrauterine pregnancy rate was achieved after isthmus-isthmus anastomoses. Unfortunately only four of 27 Pomeroy sterilizations, three of five ring or clip sterilizations, and one tubal coagulation sterilization could be reversed in this manner.

Results of fimbriectomy were poor, since only one of six patients achieved an intrauterine pregnancy. These patients were poorly selected, and none satisfied the criteria of Novy,<sup>15</sup> who advised a tubal length of at least 8 cm with an ampullary width of 1 cm as minimal measurements before carrying out a salpingostomy. He also suggested that a rugal pattern be seen at the hysterosalpingogram, and that there be minimal peritubal adhesions present. With these criteria, he was able to achieve an intrauterine pregnancy rate of 40%.

Fifteen patients had a reimplantation procedure carried out to reverse a previous extensive unipolar coagulation sterilization. Although a 60% intrauterine pregnancy rate was achieved in these 15 patients, it is not considered to be the procedure of choice. An attempt should be made to carry out a cornual anastomosis on these patients by shaving down on the intramural portion of the tube until a patent segment is found. A subsequent anastomosis leaves a longer tube, with at least as good a chance for achieving an intrauterine pregnancy, and allows the patient to be able to deliver normally without having a cesarean section as would be required in the reimplantation group. Gomel<sup>13</sup> has advocated this technique and has constructed a special blade to enable easier access to the intramural portion of the fallopian tube. A tubal reimplantation has not been carried out for over 2 years in our series of cases.

Our pregnancy rate following reversal of unipolar tubal coagulations (60%) was similar to that of Rock et al.<sup>16</sup>

The six ectopic pregnancies in this series (7%) oc-

curred only in ampullary-isthmic and ampullary-cornual anastomoses. In both these situations there is a marked disparity in the caliber of the lumen in each of the segments to be reanastomosed. Moreover, four of the six ectopic pregnancies had a previous postpartum sterilization in which a large segment of the tube had been removed. It is believed that the sterilizations should ideally be carried out as an interval procedure, but if it is necessary to proceed in the postpartum period or during a cesarean section, only a small portion of isthmus should be removed so that an isthmic-isthmic reanastomosis would be possible in event that a reversal is necessary in the future.

In the past, sterilizations were often extremely destructive operative procedures, with little thought given to the possibility of a subsequent reversal. Many tubes were totally cremated by multiple burns with unipolar cautery. In addition, excessive destruction was carried out with the application of numerous clips, excision of large segments of tubes, and excision of the fimbriated ends of the tubes. The only concern of the surgeon was to prevent the possibility of a failed sterilization and a resulting unwanted pregnancy.

It is estimated that 1% of women will seek a reversal of a previous sterilization. Therefore it is incumbent on those of us who perform sterilizations to seek out the high-risk group of patients who may request a future reversal and to counsel this group appropriately. For example, the young patient of low parity in an unstable marriage who is under significant stress would be a high-risk candidate for subsequent reversal of a sterilization. She should be offered an alternate form of contraception, with the assurance of the back-up of an abortion should the contraceptive method fail.

The ideal sterilization is one that provides the lowest failure rate and the maximal possibility of a future reversal. It is suggested that a single burn technique with use of bipolar cautery or clip application in the mid-isthmic portion of the fallopian tube satisfies these criteria and would be the ideal method of sterilization in the woman under 40 years of age. Sterilization should be looked on as a permanent procedure, but in view of the significant and radical changes occurring in our

society, we must make allowances for possible regret and change of mind on the part of our sterilized patients. The suggested methods of sterilization would allow a subsequent isthmic-isthmic tubal anastomosis and offer approximately 80% of these patients the prospect of achieving a live birth following reversal surgery.

### Addendum

Since presentation of the paper, two more of the 89 patients studied in detail have achieved an ongoing intrauterine pregnancy.

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# PAPERS OF THE SOCIETY FOR GYNECOLOGIC INVESTIGATION

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## Variability and selectivity of anterior pituitary response to dopamine agonists throughout the normal menstrual cycle

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To clarify whether there is a variation of dopamine effect throughout the normal menstrual cycle, 24 studies were performed during the follicular, periovulatory, and luteal phase in seven ovulatory women. The subjects were studied for 24 hours after receiving two different dopamine agonists, 2.5 mg of bromocriptine in one cycle and 50 µg of pergolide in a subsequent cycle. Baseline plasma luteinizing hormone, follicle-stimulating hormone, prolactin, and thyrotropin were followed through time, and the dynamic responses to gonadotropin-releasing hormone and thyrotropin-releasing hormone before and at 6 and 22 hours after medication were studied. Since the results obtained with both agonists were similar, the data have been combined in a single group. Baseline luteinizing hormone levels (but not follicle-stimulating hormone) were significantly suppressed ( $p < 0.01$ ) during the follicular phase only, and the plasma luteinizing hormone and follicle-stimulating hormone response to gonadotropin-releasing hormone was not affected by the agonists in any of the three cycle phases. Baseline plasma prolactin was suppressed equally ( $p < 0.005$ ) in all phases of the cycle, and the response to thyrotropin-releasing hormone was similarly suppressed in all phases only at 6 hours ( $p < 0.002$ ). Baseline thyrotropin also was suppressed ( $p < 0.01$ ) in all phases but the degree of inhibition was greater in the luteal than in the follicular phase ( $p < 0.05$ ). The response to thyrotropin-releasing hormone was inhibited, with the smallest response seen at 22 hours ( $p < 0.01$ ). In conclusion, these results suggest that the modulatory effect of dopamine on pituitary hormone secretion is variable and selective throughout the normal menstrual cycle. The greatest inhibition is on prolactin release, which is similar in all phases, followed by thyrotropin, which is greater in the luteal phase, and then by luteinizing hormone in the follicular phase only; it has no effect on follicle-stimulating hormone release. (AM J OBSTET GYNECOL 1986;154:362-7.)

**Key words:** Pituitary, dopamine agonists, menstrual cycle

The suppressive effect of dopamine and dopamine agonists on prolactin secretion has been well established.<sup>1-9</sup> However, the gonadotropins response to these agents has been the subject of some controversy. Dopamine administered for 4 hours was shown by Leblanc et al.<sup>1</sup> to significantly suppress luteinizing hormone but not follicle-stimulating hormone secretion in women studied in the early follicular phase. Kaptein et al.<sup>2</sup> reported that the administration of dopamine for 48 consecutive hours to normal men had inhibited transiently

both luteinizing hormone and follicle-stimulating hormone secretion, with a definitive escape during continued dopamine infusion. Leebaw et al.<sup>3</sup> failed to demonstrate an inhibition on basal luteinizing hormone and follicle-stimulating hormone secretion, but they reported a blunted luteinizing hormone response to gonadotropin-releasing hormone in normal men receiving a dopamine infusion. L-Dopa, which is centrally decarboxylated to dopamine, has been shown by Lachelin et al.<sup>4</sup> to suppress only luteinizing hormone secretion when given in the early follicular phase.<sup>4</sup> However, bromocriptine administration resulted in a significant inhibition of both luteinizing hormone and follicle-stimulating hormone secretion in women with amenorrhea and hyperprolactinemia.<sup>4</sup> Yet Evans et al. could not confirm these findings in similar patients treated with bromocriptine<sup>5</sup> or in normal men treated with pergolide, another dopamine agonist.<sup>6</sup> Furthermore, Martin et al.<sup>7</sup> subsequently showed a stimulatory effect of

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**Table I.** Pretreatment baseline hormone levels (mean  $\pm$  SEM)

	<i>Follicular</i>	<i>Preovulatory</i>	<i>Luteal</i>
Estradiol-17 $\beta$ (pg/ml)	88 $\pm$ 18	203 $\pm$ 34*	214 $\pm$ 27*
Progesterone (ng/ml)	0.5 $\pm$ 0.1	1.4 $\pm$ 1.0	10 $\pm$ 1.9†
Prolactin (ng/ml)	16.2 $\pm$ 3.5	16.9 $\pm$ 3.4	22 $\pm$ 4.6
Thyrotropin ( $\mu$ U/ml)	2.0 $\pm$ 0.2	1.3 $\pm$ 0.3	1.2 $\pm$ 0.1
Luteinizing hormone (mIU/ml)	11.3 $\pm$ 0.5	21 $\pm$ 2.1 <sup>+</sup>	11.8 $\pm$ 5.6
Follicle-stimulating hormone (mIU/ml)	6.5 $\pm$ 0.7	6.4 $\pm$ 1.2	4.7 $\pm$ 1.4

All values are within the normal range for each phase of the menstrual cycle.

\*p < 0.05 versus follicular phase.

†p < 0.02 versus follicular phase.

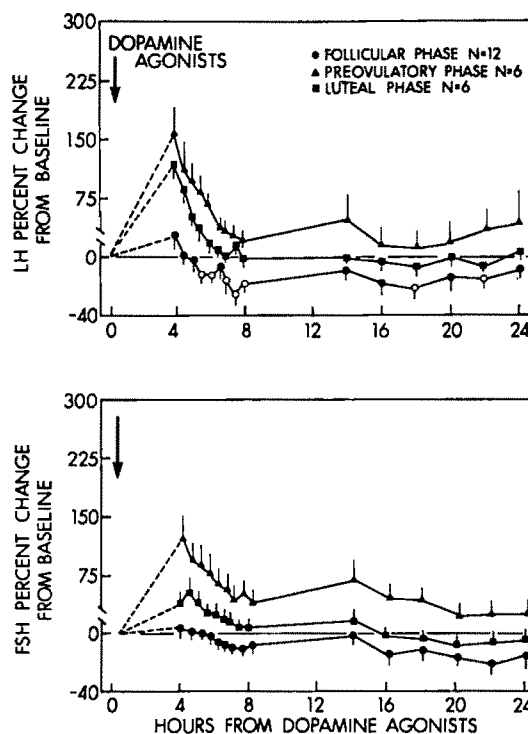
bromocriptine on luteinizing hormone levels in normal women studied within 5 days of midcycle luteinizing hormone peak. Thus the inhibitory effect, or the lack of it, of dopamine agonists on serum luteinizing hormone levels may depend on the degree of the endogenous dopamine activity, which may vary throughout the menstrual cycle.

The study reported herein was undertaken to investigate whether or not there is a different dopamine activity in the follicular, preovulatory, and luteal phases of normal menstrual cycles. For that purpose, two dopamine agonists (bromocriptine and pergolide mesylate) were given and the effect on baseline and dynamic secretion of luteinizing hormone, follicle-stimulating hormone, prolactin, and thyrotropin were determined.

#### Material and methods

Seven ovulatory women with menstrual cycles between 28 and 30 days in length participated in this investigation, and 24 studies were completed. Six of these women were studied twice in the follicular phase, between days 2 and 8 of two consecutive cycles, receiving either 2.5 mg of bromocriptine or 50  $\mu$ g of pergolide. The seventh subject as well as two of the first six women were later studied twice in the preovulatory period on days 12 to 14 and again in the midluteal phase on days 20 to 22 of two cycles in which they received the same dopamine agonists.

All women were admitted to the Clinical Research Center at 7 AM, at which time a 21-gauge butterfly needle with a heparin lock attached to maintain patency was inserted into an arm vein. Subjects received a low-fat diet, and activity and sleep were permitted ad libitum. Baseline blood samples were obtained at 7:30 AM and 8 AM. Pretreatment provocative testing was per-



**Fig. 1.** Mean ( $\pm$ SEM) percent change from baseline of plasma luteinizing hormone and follicle-stimulating hormone throughout the normal menstrual cycle following the administration of dopamine agonists. Open symbols represent significance of p < 0.01.

formed at 8 AM with gonadotropin-releasing hormone, 100  $\mu$ g, and thyrotropin-releasing hormone, 500  $\mu$ g by intravenous bolus; blood samples were collected at 30, 60, and 120 minutes thereafter. At 10 AM all subjects received the dopamine agonist orally (either 2.5 mg of bromocriptine or 50  $\mu$ g of pergolide). Further blood samples were subsequently drawn every 30 minutes between 2 PM and 6 PM, at which time a second stimulation test with gonadotropin-releasing hormone/thyrotropin-releasing hormone, as described above, was performed. Following this 240-minute time period, sampling continued every 2 hours throughout the night. On the morning of day 2 a third and final gonadotropin-releasing hormone/thyrotropin-releasing hormone test was administered, which was 22 hours after administration of the dopamine agonist.

Blood samples were centrifuged, and plasma was separated and stored at  $-20^{\circ}$  C until assayed. All samples were analyzed for prolactin, thyrotropin, luteinizing hormone, and follicle-stimulating hormone by radioimmunoassay as previously described.<sup>10,11</sup> Additionally, samples from 8 AM on day 1 and 2 were assayed for estradiol-17 $\beta$  and progesterone. For statistical comparisons, individual values and net increase of the area under the curve following gonadotropin-releasing hormone/thyrotropin-releasing hormone stimulations



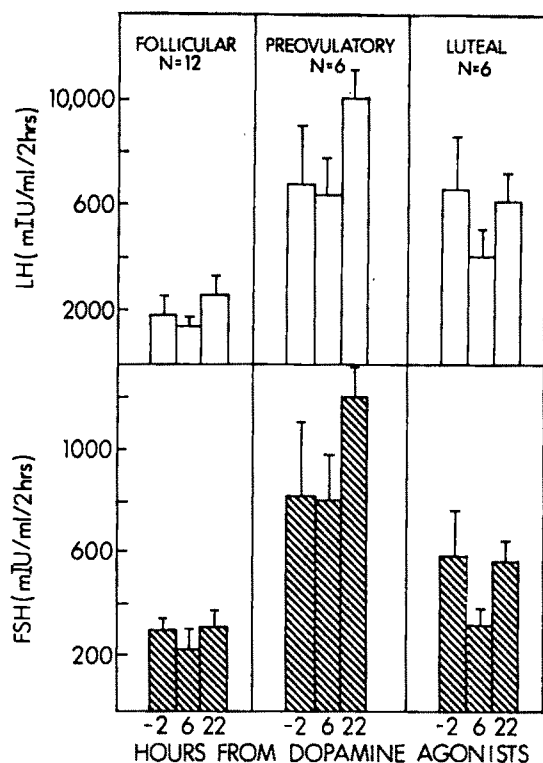


Fig. 2. Net increase of plasma luteinizing hormone and follicle-stimulating hormone (mean  $\pm$  SEM) levels throughout the normal menstrual cycle following gonadotropin-releasing hormone administration before and 6 and 22 hours after administration of dopamine agonists.

were converted to their natural logarithms.<sup>15</sup> Analysis was first performed by two-way analysis of variance, and the geometric means were compared by multiple paired *t* tests adjusted for multiple comparisons. Baseline values were compared by *t* test, and significance level was established at  $p < 0.05$ .

### Results

Mean pretreatment and posttreatment baseline values of all hormones and the response to gonadotropin-releasing hormone and thyrotropin-releasing hormone in each phase of the cycle did not differ significantly between bromocriptine and pergolide study, and therefore the results were combined in a single group. Mean ( $\pm$  SEM) baseline estradiol-17 $\beta$  levels rose significantly ( $p < 0.01$ ) from the follicular to the periovulatory period but did not change further in the luteal phase. Plasma progesterone rose significantly only in the luteal phase ( $p < 0.02$ ). Serum luteinizing hormone levels in the preovulatory phase were significantly greater ( $p < 0.002$ ) than that in the follicular and luteal phase (Table I).

Following the administration of the agonists, baseline plasma luteinizing hormone levels were suppressed only in the follicular phase. The inhibition became sig-

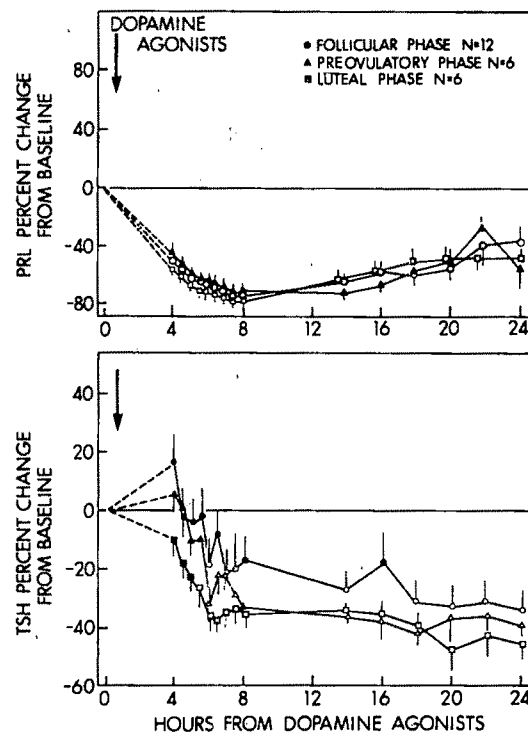


Fig. 3. Mean ( $\pm$  SEM) percent change from baseline of plasma prolactin and TSH values throughout the normal menstrual cycle following the administration of dopamine agonists. Open symbols represent significance of at least  $p < 0.01$ .

nificant ( $p < 0.01$ ) 5½ hours after the agonists were administered (Fig. 1). The preovulatory and luteal phases were notable in that no significant suppression of luteinizing hormone levels below the pretreatment baseline occurred at any point in time. A small and not significant suppression of serum follicle-stimulating hormone was observed in the follicular phase only (Fig. 1).

Plasma luteinizing hormone and follicle-stimulating hormone response to gonadotropin-releasing hormone stimulation were not altered by the dopamine agonist in any phase of the menstrual cycle (Fig. 2). However, the area under the curve of luteinizing hormone and follicle-stimulating hormone response to gonadotropin-releasing hormone given before the agonists was significantly greater in the preovulatory and luteal phase ( $p < 0.01$ ) than in the follicular phase (Fig. 2).

Dopamine agonists induced a significant ( $p < 0.0005$ ) reduction of baseline plasma prolactin levels, to a maximum of 81% below baseline at 6 hours. Although there was a tendency of prolactin values to return to baseline after 20 hours, nonetheless they remained significantly lower ( $p < 0.02$ ) (Fig. 3). This pattern of prolactin inhibition was identical for all three phases of the menstrual cycle. A similar pattern of inhibition was induced on plasma thyrotropin secretion in the follicular, preovulatory, and luteal phase (Fig. 3). Significant serum

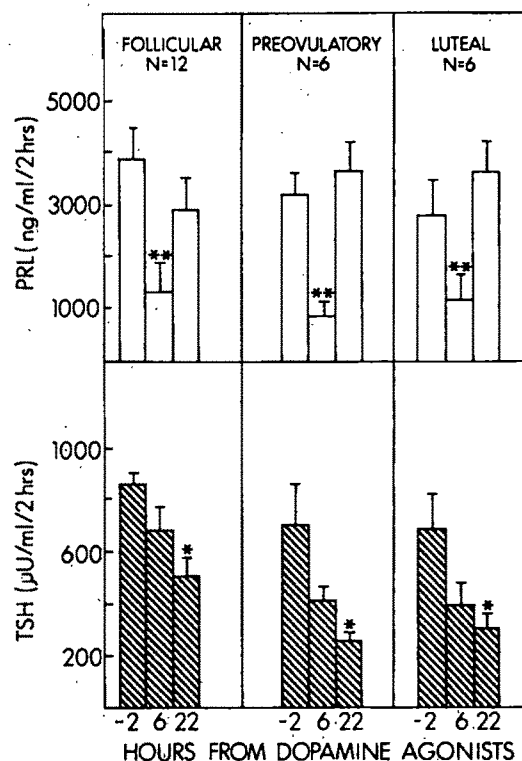
thyrotropin suppression ( $p < 0.01$ ) occurred at about 6 hours and remained significantly inhibited for the rest of the 24 hours. However, the integrated area of inhibition from 4 to 8 hours after the agonists were administered was significantly larger ( $p < 0.05$ ) in the luteal than in the follicular phase ( $112.3\% \pm 31\%$  versus  $35.7\% \pm 30\%$ ).

Net secretions of prolactin during the 2-hour stimulation with thyrotropin-releasing hormone were inhibited at 6 hours in the follicular ( $p < 0.002$ ), preovulatory ( $p < 0.001$ ), and luteal ( $p < 0.05$ ) phases (Fig. 4). In all three phases the prolactin response to the thyrotropin-releasing hormone administered 22 hours after the dopamine agonists was similar to that seen during pretreatment and significantly greater ( $p < 0.01$ ) than that seen after the second thyrotropin-releasing hormone.

The response of thyrotropin to thyrotropin-releasing hormone exhibited a pattern of increasing suppression throughout the 24-hour study period in all phases. Nonetheless, statistically significant diminution ( $p < 0.02$ ) of the 2-hour net secretion occurred only at 22 hours (Fig. 4).

# Comment

The investigation has demonstrated that dopamine agonists selectively induced a significant inhibition of baseline luteinizing hormone but not of follicle-stimulating hormone in the early follicular phase only. Furthermore, both luteinizing hormone and follicle-stimulating hormone levels in the preovulatory phase remained above pretreatment values for the entire study, demonstrating that the sensitivity of the pituitary to the inhibitory effect of dopamine agonists on baseline luteinizing hormone release decreases as the sensitivity of the gonadotrops to gonadotropin-releasing hormone increases. Thus the conflicting published results<sup>1-7</sup> can be explained by the fact that similar studies were performed at different times of the cycle when there is a changing sensitivity to dopamine. During the follicular phase the release of baseline luteinizing hormone is inhibited,<sup>1,4</sup> but during the preovulatory and luteal phase it is not.<sup>5,7</sup> Thus a decrease in pituitary sensitivity to dopamine agonists in the preovulatory and luteal phase seems to occur, since in no case should there have been a total absence of luteinizing hormone suppression. Alternatively, and in agreement with Martin et al.,<sup>7</sup> one could postulate that a preferential action of the dopamine agonist on a central presynaptic receptor might inhibit endogenous dopamine action at midcycle and thereby remove the suppressive influence on luteinizing hormone secretion. Such modulation could be produced by the increasing estrogen and progesterone concentrations. This view is at variance with previous reports that demonstrated a maximal inhibi-



**Fig. 4.** Net increase of plasma prolactin and thyrotropin (mean  $\pm$  SEM) throughout the normal menstrual cycle following thyrotropin-releasing hormone administration before and 6 and 22 hours after administration of dopamine agonists. \* $p < 0.02$ , \*\* $p < 0.01$ .

tion of luteinizing hormone on day 14 of the cycle following the administration of dopamine.<sup>13</sup> In view of the failure of dopamine agonists to alter pituitary sensitivity to exogenous gonadotropin-releasing hormone, it seems likely that the selective follicular phase suppression of circulating luteinizing hormone baseline levels by dopamine is mediated largely via a central effect on endogenous gonadotropin-releasing hormone pulses. Although reaching similar conclusions, others have reported a blunted luteinizing hormone response to gonadotropin-releasing hormone during dopamine infusion to normal women studied in the follicular phase.<sup>3,13</sup> It could be also possible that the effects of dopamine agonists cannot be equated with those induced by dopamine itself. It is unclear whether the lack of significant inhibition of plasma follicle-stimulating hormone in the follicular phase was due to the use of an agonist or to the prolonged half-time of follicle-stimulating hormone. Thus it can be concluded that dopamine may play a limited modulatory effect in the release of luteinizing hormone and only in the follicular phase.

The selectivity of dopamine agonists' modulation on pituitary hormone release during the menstrual cycle is further demonstrated by the response on prolactin

and thyrotropin release. Baseline plasma prolactin was uniformly suppressed about 80% throughout the menstrual cycle, lasting for at least 24 hours and therefore abolishing also the sleep-entrained rise of prolactin. Thus it can be suggested that a dopaminergic mechanism may be involved, at least in part, in the nocturnal rise of prolactin and thyrotropin.<sup>14, 15</sup> The inhibition of prolactin response to exogenous thyrotropin-releasing hormone during a period of continued dopaminergic suppression of tonic prolactin release (6 hours) suggests that the effect is largely occurring at the pituitary level.

Inhibition of thyrotropin release was larger during the luteal phase than during the follicular phase ( $p < 0.05$ ), again indicating a variable dopamine activity throughout the normal menstrual cycle.<sup>16, 17</sup> Furthermore, the degree of inhibition seems to be time dependent, since plasma thyrotropin released to thyrotropin-releasing hormone administration decreased in a stepwise fashion and became significant ( $p < 0.02$ ) 22 hours later. This inhibition occurs at a time when prolactin, which is the main pituitary hormone controlled by dopamine inhibition, is again responding normally to exogenous thyrotropin-releasing hormone. The pattern of decline in thyrotropin release could not be explained either by an exhaustion of endogenous thyrotropin<sup>18</sup> or by a negative feedback of increasing levels of thyroxine.<sup>19</sup> It is possible that it is the result of down regulation of pituitary thyrotropin-releasing hormone receptors produced by the dopamine agonists. Alternatively, the thyrotrops may exhibit a greater binding affinity for the dopamine agonists than do the lactotrops. It is of interest that it has been reported that the administration of pergolide to normal men resulted in progressive loss in the inhibition of thyrotropin response to thyrotropin-releasing hormone in spite of continuing suppression of prolactin release.<sup>7</sup> Thus this sex difference could be the result of estrogen and progesterone modulation of dopamine function.<sup>20</sup> It seems clear that dopamine exerts a more direct influence in the secretion of prolactin and thyrotropin than on gonadotropins. The changing steroid milieu throughout the menstrual cycle appears to have no effect on the constant inhibition of prolactin secretion, thus preventing large fluctuations of prolactin which could interfere with the normal process of ovulation and corpus luteum function. The physiologic importance of a lesser thyrotropin inhibition during the follicular phase remains to be elucidated.

In conclusion, we have demonstrated the variability and selectivity of pituitary hormone release to dopamine agonists throughout the menstrual cycle. Prolactin release was shown to be equally inhibited in all phases, followed by a lesser suppression on thyrotropin release but selectively greater one in the luteal phase. The inhibition of luteinizing hormone release occurred

in the follicular phase only and no significant effect on follicle-stimulating hormone release could be demonstrated.

We wish to thank the National Pituitary Agency, Bethesda, Maryland, for their generosity in providing the reagents for radioimmunoassay of luteinizing hormone, follicle-stimulating hormone, thyrotropin, and prolactin.

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## Inhibition of gonadotropin-releasing hormone receptors in rat anterior pituitary monolayer cell cultures by danazol

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To study possible cellular antigonadotropic effects of danazol, monolayer cultures of anterior pituitary cells from immature female rats were treated with danazol. Measurements of luteinizing hormone release in response to  $10^{-8}$  mol/L gonadotropin-releasing hormone challenge and iodine 125-labeled gonadotropin-releasing hormone binding activity were done after exposure to increasing concentrations of danazol and for increasing lengths of time. It was found that luteinizing hormone secreted by pituitary cells in response to gonadotropin-releasing hormone was inhibited after danazol treatment in a dose- and time-dependent manner when compared to controls. Also, a 45% decrease in gonadotropin-releasing hormone receptor binding capacity was observed in pituitary cells cultured in the presence of increasing concentrations of danazol in the range of  $10^{-8}$  to  $10^{-4}$  mol/L when compared to controls. Furthermore, exposure to danazol for 25 to 96 hours caused a marked decrease in gonadotropin-releasing hormone binding activity ( $p < 0.005$ ). Under these experimental conditions danazol treatment decreased the pituitary receptors for gonadotropin-releasing hormone in a dose- and time-dependent manner. Scatchard analysis of saturation curves for the binding of gonadotropin-releasing hormone to cellular gonadotropin-releasing hormone receptors indicated that the observed decrease in gonadotropin-releasing hormone binding in the danazol-treated group was due to a change in the number of gonadotropin-releasing hormone binding sites rather than a change in the affinity. It is therefore concluded that the antigonadotropic activity of danazol appears to be related to a decrease in gonadotropin-releasing hormone receptors in the pituitary. (*AM J OBSTET GYNECOL* 1986;154:367-72.)

**Key words:** Gonadotropin-releasing hormone receptor, danazol effect, pituitary cell culture, luteinizing hormone

Danazol, a synthetic heterocyclic steroid, chemically related to  $17\alpha$ -ethinyl testosterone, has received wide attention because of its various endocrine properties.<sup>1,2</sup> Since the introduction of danazol in 1971 the medical management of endometriosis has been significantly advanced. There are at least four pharmacologic mechanisms by which danazol might exert its therapeutic effects: (1) direct inhibition of gonadotropin-releasing hormone (GnRH)<sup>3</sup> and gonadotropin secretion,<sup>4</sup> (2) interaction with intracellular androgen and

progesterone receptors,<sup>5,6</sup> (3) direct inhibition of steroidogenesis, and (4) alteration of endogenous steroid metabolism.<sup>7</sup>

Our previous studies, which used pituitary cells in culture as a model system, have shown that one of the effects of danazol is directly antigonadotropic, inhibiting the responsiveness of the anterior pituitary with respect to GnRH-induced luteinizing hormone release.<sup>8</sup> The purpose of the present study was to further investigate the mechanism of this inhibition to determine whether the antigonadotropic activity of danazol is related to alteration in the number of GnRH receptors in the pituitary.

### Material and methods

Weanling 21-day-old female Sprague-Dawley rats (Spartan Farms, Hazlett, Michigan) were used in this

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study. Synthetic GnRH was a gift from Warner-Lambert Parke-Davis Co. (Detroit, Michigan). Reagents for radioimmunoassay were generously provided by the Hormone Distribution Program of the National Institute of Arthritis, Diabetes, and Kidney Diseases. Reagents for cell culture were obtained from Grand Island Biological Company (Grand Island, New York). The nondegradable analog of GnRH, desgly<sup>10</sup>[D-ala<sup>6</sup>]GnRH ethylamide, was purchased from Beckman Instruments, Inc. (Palo Alto, California). Lactoperoxidase, Dowex 50-W, and CM cellulose were obtained from Sigma Chemical Company (St. Louis, Missouri), glucose oxidase from Miles Laboratories, Inc., (Elkhart, Indiana), and iodine 125-labeled sodium from Amersham Corporation (Arlington Hts, Illinois). Danazol was a gift from Sterling-Winthrop Research Institute (Rensselaer, New York).

**Anterior pituitary cell culture.** The cell cultures were prepared by the procedure described by Vale et al.<sup>9</sup> with minor modifications. Briefly, anterior pituitaries were removed from immature female rats and were placed in Ca<sup>2+</sup>- and Mg<sup>2+</sup>-free Earle's balanced salt solution. After mincing and rinsing with Earle's solution, the tissue was incubated at 37° C with stirring in Earle's solution containing 2% bovine serum albumin (crystalline, Miles Laboratories, Elkhart, Indiana), 0.3% collagenase (type I, Worthington Biochemical Corporation, Freehold, New Jersey), and 0.1% hyaluronidase (Worthington) for 60 to 75 minutes followed by treatment with 0.25% pancreatin (Grand Island Biological) for 15 minutes. After three rinsings the cells were suspended in medium at a final concentration of  $5 \times 10^5$  cells per milliliter; 2.0 ml of this suspension ( $10^6$  cells) were plated to tissue culture dishes (35 × 10 mm, Falcon Plastics, Oxnard, California) and maintained at 37° C in a water-saturated atmosphere of 95% air/5% carbon dioxide. Cell attachment occurred 48 hours after plating. The medium used for culture was Dulbecco's modified Eagle's medium supplemented with 10% horse serum, 2.5% fetal calf serum, 0.1 mmol/L non-essential amino acids, glutamine, gentamicin (50 µg/ml), and mycostatin (10 IU/ml) (supplemented Dulbecco's modified Eagle's medium). The sera were previously treated with dextran coated charcoal to remove endogenous steroids.

After 2 days of culture the medium was replaced with supplemented Dulbecco's modified Eagle's medium containing danazol. Danazol was dissolved in 70% ethanol; final concentration of ethanol was 0.5%, which was also included in the control cultures. For luteinizing hormone release studies, after incubation for indicated times with the test substance, the cultures were washed with medium and challenged for 4 hours in Dulbecco's modified Eagle's medium containing  $10^{-8}$  mol/L of GnRH. At the end of this incubation period the media

were collected and centrifuged for 10 minutes at  $1500 \times g$ . The supernatants were assayed for luteinizing hormone by radioimmunoassay<sup>10</sup> with use of a rat luteinizing hormone preparation (NIAMDD-RP-1) as standard.

**Binding assay of gonadotropin-releasing hormone receptors.** At the end of incubations, as described in each experimental protocol in the figure legends, the cultured cells were washed twice with Dulbecco's modified Eagle's medium, scraped free with a rubber policeman, and transferred to 12 × 75 mm glass tubes at a concentration of approximately  $2 \times 10^6$  cells per tube. Preparation of radioiodinated D-ala<sup>6</sup> GnRH for use as radioligand was performed by the lactoperoxidase method.<sup>11</sup> The specific activity of the labeled peptide ranged from 850 to 1500 µCi/µg.

The assay for GnRH receptors was performed as follows<sup>12</sup>: 50,000 cpm of [<sup>125</sup>I]D-ala<sup>6</sup> GnRH (approximately  $10^{-11}$  mol/L) were incubated with approximately  $2 \times 10^6$  pituitary cells for 80 minutes at 22°. Nonspecific binding was determined in the cells incubated with tracer and excess unlabeled ligand ( $2 \times 10^{-5}$  mol/L). Specific binding was assessed by subtracting nonspecific binding from the total binding. Incubations were performed in a total volume of 0.3 ml of assay buffer (Dulbecco's modified Eagle's medium, 0.1% bovine serum albumin, and 25 mmol/L of N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (Hepes) with 0.5 mmol/L of bacitracin, pH 7.4) and terminated with 2 ml of ice-cold phosphate-buffered saline solution (pH 7.4) followed by immediate filtration under vacuum through glass fiber filters (Whatman GF/C, Whatman, Inc., Clifton, New Jersey) presoaked in 1% bovine serum albumin in phosphate-buffered saline solution, in a multiphase holder. The filters with pituitary cells were washed three times with 4 ml of phosphate-buffered saline solution, and bound radioactivity was determined in a gamma spectrometer. To study the effect of danazol on GnRH receptor content and affinity, pituitary cells after danazol treatment were incubated with 50,000 cpm of radioiodinated D-ala<sup>6</sup> GnRH and increasing concentrations of unlabeled analog from 0.25 to 2.25 mmol/L in the absence or presence of a 1000-fold excess of unlabeled analog. The amount of GnRH bound to the cells was determined at each point.

**Assay of protein synthesis.** After cell attachment, media were replaced with fresh supplemented Dulbecco's modified Eagle's medium containing 10 µCi of tritiated L-leucine (110 µCi/mmol, New England Nuclear, Boston, Massachusetts) and incubated with test substances for the indicated time. At the end of the radiolabeling period, media were removed, cells were rinsed five times with serum-free media and dissolved in 0.1N sodium hydroxide. Aliquots were neutralized with 0.1N hydrochloric acid, and after addition of 0.1

**Table I.** Effect of increasing concentrations of danazol on the in vitro responsiveness of pituitary cells to gonadotropin-releasing hormone

	Luteinizing hormone secreted ( $\mu\text{g/ml} \pm \text{SEM}$ )
Control	$2.38 \pm 0.20$
Danazol ( $\mu\text{mol/L}$ )	
0.1	$2.01 \pm 0.37$
0.5	$1.42 \pm 0.10^*$
1	$1.31 \pm 0.05^\dagger$
5	$0.07 \pm 0.06^\ddagger$
10	$0.53 \pm 0.06^\ddagger$

Triplicate cell cultures were exposed to 0.1 to 10  $\mu\text{M}$  of danazol for 48 hours, and control cultures received equivalent volume of solvent (0.5% ethanol in Dulbecco's modified Eagle's medium). At the end of this period the media were replaced with Dulbecco's modified Eagle's medium containing  $10^{-8}$  mol/L of gonadotropin-releasing hormone, and incubation continued for 4 hours. Luteinizing hormone released into the medium was measured by radioimmunoassay.

\* $p < 0.010$ .

$^\dagger p < 0.005$ .

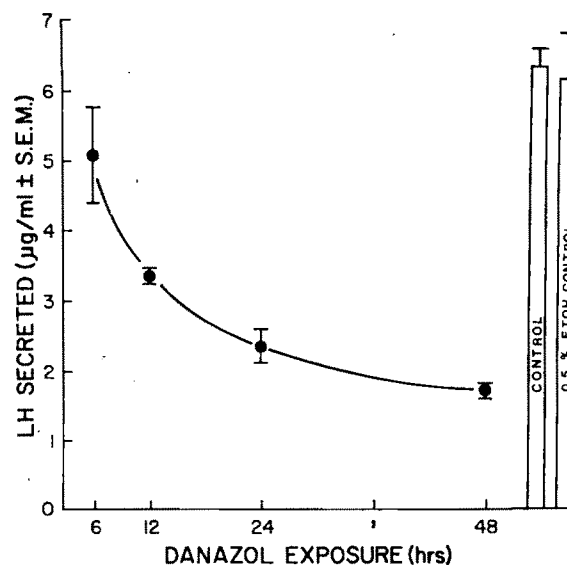
$^\ddagger p < 0.001$ .

mg of bovine serum albumin, equal volumes of 20% trichloroacetic acid containing 2% phosphotungstic acid and 0.1% L-leucine were added. After 60 minutes on ice, samples were centrifuged at  $3000 \times g$  for 20 minutes. The pellets were washed five times by dissolving in 1N sodium hydroxide followed by precipitation with 1N hydrochloric acid. The final pellet was dissolved in NCS tissue solubilizer (Amersham Corporation, Arlington Heights, Illinois) and counted in a liquid scintillation counter after the addition of scintillation cocktail (composed of 4 gm of diphenyloxazole and 50 mg of 1,4-bis[2-(5-phenyloxazolyl)]benzene; phenyloxazolylphenyloxazolyl-phenyl (Popop) in 1 L of toluene).

**Protein determination.** Cellular protein content was determined by the colorimetric procedure of Lowry et al.<sup>13</sup> with use of bovine serum albumin as standard. Cell protein content in these studies did not vary significantly in control versus danazol-treated cultures.

## Results

**Effect of danazol exposure on luteinizing hormone response to gonadotropin-releasing hormone.** To determine the effect of danazol pretreatment on the sensitivity of the pituitary cells to GnRH for luteinizing hormone release, cultures were exposed to 0.1 to 10  $\mu\text{mol/L}$  of danazol for 48 hours, after which the medium was replaced with Dulbecco's modified Eagle's medium, and the incubation was continued with  $10^{-8}$  mol/L of GnRH for 4 hours. The amount of luteinizing hormone released into the medium was assayed by ra-

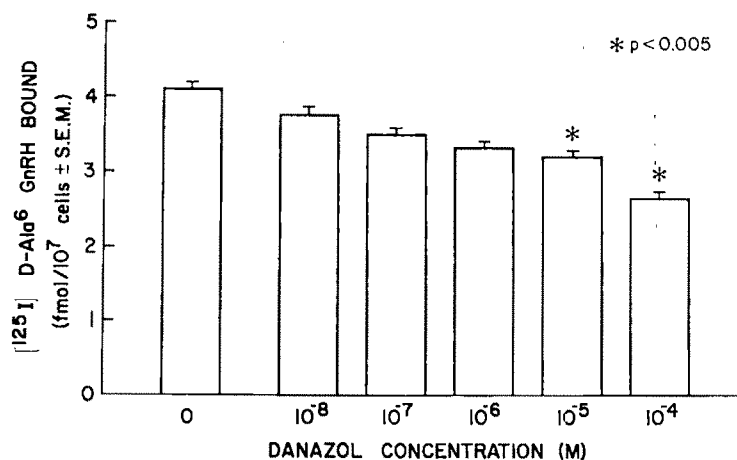


**Fig. 1.** Time course of the effect of danazol exposure on the responsiveness of pituitary cell cultures to GnRH. After cell attachment (48 hours), cells were incubated with  $5 \times 10^{-6}$  mol/L of danazol for increasing lengths of time from 6 to 48 hours. Control cultures were exposed to diluent. At the end of each exposure time, media were replaced with Dulbecco's modified Eagle's medium containing  $10^{-8}$  mol/L of GnRH and incubation continued for 4 hours. After this incubation period, media were removed and assayed for luteinizing hormone (LH) content by radioimmunoassay.

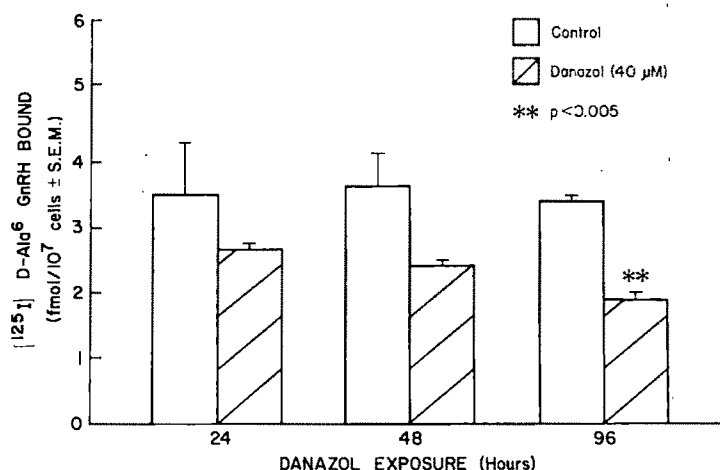
dioimmunoassay. It can be observed in Table I that danazol treatment of the pituitary cells significantly inhibited the GnRH-stimulated luteinizing hormone release in a dose-dependent manner when compared to controls.

The time course for danazol effect on pituitary cell responsiveness to GnRH with respect to luteinizing hormone release was then examined (Fig. 1). Cultures were exposed to  $5 \times 10^{-6}$  mol/L of danazol for increasing lengths of time, from 6 to 48 hours, after which luteinizing hormone released in response to a  $10^{-8}$  mol/L of GnRH challenge was measured. The results demonstrate a significant, progressive decline in luteinizing hormone secretion in response to GnRH as the duration of exposure to danazol is increased. After 48 hours of danazol exposure, luteinizing hormone response was 30% of controls ( $p < 0.005$ ). Thus pretreatment with danazol causes a decreased responsiveness to GnRH for luteinizing hormone release in a time and concentration dependent manner.

**Effect of danazol pretreatment on GnRH receptor.** Further experiments were performed to determine whether the decreased responsiveness of the pituitary cells for luteinizing hormone release may be due to loss of pituitary receptors for GnRH. In the first experiment the cultures were treated with increasing concentrations of danazol ( $10^{-8}$  to  $10^{-4}$  mol/L), after which



**Fig. 2.** Effect of danazol on GnRH binding activity. Pituitary cell cultures were incubated with increasing concentrations of danazol or control diluent, after which the cells were removed and binding of [<sup>125</sup>I]D-al<sup>6</sup> GnRH was determined as described under Materials and methods (\**p* < 0.005 versus control).

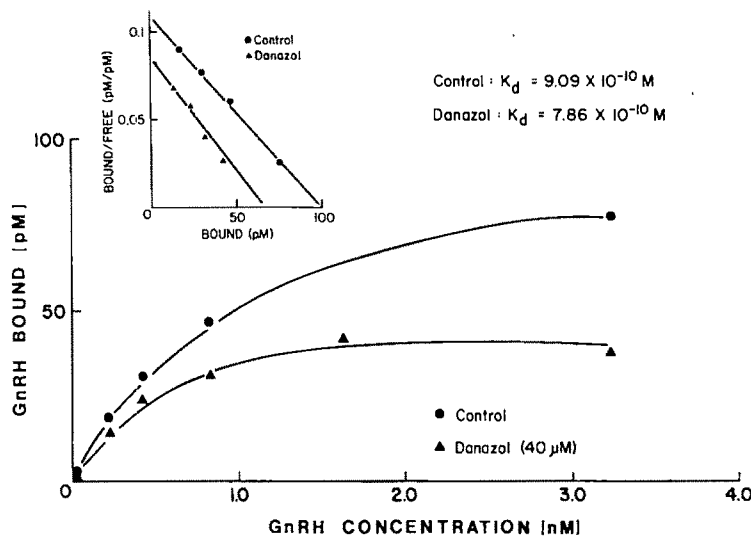


**Fig. 3.** Effect of increasing danazol exposure time on GnRH binding activity. Pituitary cell cultures were incubated with 40 μmol/L of danazol or control diluent for increasing lengths of time as indicated. At the end of each time period, cells were collected and GnRH binding studies were performed as described in Materials and methods (\*\**p* < 0.005 versus control at the same time point).

the cells were collected and binding of [<sup>125</sup>I]D-al<sup>6</sup> GnRH was determined. As seen in Fig. 2, there appears to be a dose-related inhibitory effect of danazol on GnRH binding activity, with a significant decrease (*p* < 0.005) exhibited in those cultures exposed to 10<sup>-5</sup> and 10<sup>-4</sup> mol/L of danazol.

The effect of danazol exposure on GnRH binding capacity by pituitary cells was then determined. Cultures were incubated with 40 μmol/L of danazol for 24, 48, and 96 hours, and the cells were collected and GnRH binding assays were performed. As indicated in Fig. 3, there is a progressive decrease in GnRH binding with longer exposure to danazol, with a significant (*p* < 0.005) decrease in binding activity in those cultures pretreated with danazol for 96 hours when compared to controls.

To determine whether the effect of danazol on GnRH receptors was due to a change in the binding affinity, control and danazol-treated cells were assayed for GnRH binding activity with [<sup>125</sup>I]D-al<sup>6</sup> GnRH (~10<sup>-11</sup> mol/L) and increasing concentrations of unlabeled analog to approach saturation of the GnRH receptor sites (Fig. 4). These data were transformed according to Scatchard<sup>14</sup> to determine the diffusion constant and the number of binding sites. The Scatchard plots of the binding data gave two linear, parallel lines suggesting that the observed decrease in GnRH binding in danazol-treated cells was due to a change in the number of binding sites rather than a change in the affinity. For control cells, the diffusion constant was 9.09 × 10<sup>-10</sup> mol/L and the binding capacity was 100 fmol/10<sup>7</sup> cells. For danazol-treated cells the diffusion



**Fig. 4.** Saturation curve of D-ala<sup>6</sup> GnRH binding to pituitary cells maintained for 3 days in the presence or absence of 40  $\mu\text{mol/L}$  of danazol. Cell cultures were incubated with or without danazol (40  $\mu\text{mol/L}$ ) for 72 hours, then were removed from culture dishes, and binding of [<sup>125</sup>I]D-ala<sup>6</sup> GnRH was determined with the use of a fixed quantity of labeled ligand with increasing concentrations of unlabeled analog. Other experimental conditions were as described under Material and methods. A Scatchard plot of these data is shown in the inset. ●—● = Control. ▲—▲ = Danazol.  $K_d$  = Diffusion constant.

constant was  $7.86 \times 10^{-10}$  mol/L and the binding capacity was 65 fmol/ $10^7$  cells, indicating that no significant change in the equilibrium dissociation constant had occurred but rather that the receptor number had decreased.

**Effect of danazol treatment on protein synthesis.** To determine whether danazol exposure affected protein synthesis by the pituitary cell cultures, the incorporation of tritiated leucine into trichloroacetic acid precipitable proteins was measured. As can be seen in the data presented in Table II, no appreciable change in protein synthesis occurred in those cells treated with danazol. Total cellular protein content also was similar in control and danazol-treated cultures.

#### Comment

Although danazol is widely used in the clinical management of a number of gynecologic and endocrine disorders, the cellular mechanism of action of danazol is not yet understood. However, its antigonadotropic action has been widely recognized. The site of antigonadotropic action has been reported to be both at the hypothalamic<sup>15</sup> and at the pituitary<sup>16</sup> level. We have previously reported that exposure of rat anterior pituitary monolayer cell cultures to danazol in vitro caused an inhibition of the responsiveness of the cells to GnRH challenge for luteinizing hormone release in a dose-related manner.<sup>8</sup> Additionally, those studies also revealed that the inhibitory effect of danazol on luteinizing hormone release is not due to inhibition of luteinizing hormone synthesis in the cell cultures but

**Table II.** Effect of danazol on the incorporation of tritiated leucine into proteins in pituitary cell cultures

	<i>pmol of tritiated leucine incorporated mg of protein <math>\pm</math> SEM</i>
Control	13.63 $\pm$ 0.35
Danazol (mol/L)	
$10^{-7}$	14.93 $\pm$ 0.10
$10^{-6}$	12.76 $\pm$ 2.76
$10^{-5}$	14.78 $\pm$ 0.14

After cell attachment, media were replaced with fresh media containing the indicated test substances and 10  $\mu\text{Ci}$  of tritiated L-leucine, and incubation was continued for 96 hours. Media were then removed, and after four rinsings with Dulbecco's modified Eagle's medium, the cells were processed for trichloroacetic acid precipitable radioactivity as described in Material and methods.

to diminished responsiveness to GnRH. This brought up the interesting possibility that danazol acts at the pituitary level by inhibition of some step(s) involved in the action of GnRH on the pituitary. The concentration of danazol used in the present in vitro studies is slightly higher than the blood concentrations of danazol in women treated with a single 400 mg dose of the drug.<sup>17</sup> However, it is conceivable that the present concentrations may be achieved in therapeutic situations when the treatment lasts for longer periods and consists of higher doses. Thus the concentration of the danazol that produced the in vitro effect is comparable to that seen in therapeutic situations.



The present study clearly shows that exposure to danazol decreases the number of GnRH receptors in pituitary cell cultures, and this decrease is not due to a change in the affinity of GnRH-receptor interaction. The decrease in the binding of  $^{125}\text{I}$ -labeled GnRH is not due to an adverse effect of danazol on the viability of pituitary cells in culture, since no change was observed in amino acid incorporation into proteins or in total protein content in danazol-treated cells when compared to controls. That the inhibition of GnRH receptors is not due to nonspecific steroid effect of danazol is also evidenced by the fact that incubation with cholesterol did not alter GnRH binding activity when compared to controls (data not shown).

The mechanism by which danazol exposure causes a diminution in the number of GnRH receptors in the pituitary is not currently understood. A number of possibilities may account for this decrease. First, it is possible that danazol may alter the GnRH receptor turnover, so that the internalized receptors or newly synthesized GnRH receptors may not be recycled, thereby preventing their entry into plasma membranes.

A second possibility is that danazol may selectively inhibit synthesis of GnRH receptors in the gonadotrophs. Since the concentration of gonadotrope is low in relation to other cell types, it is possible that this selective inhibition of the receptor protein(s) may not be reflected when the effect of danazol was tested on the total protein content in the cell. Such a possibility is therefore still likely although no change in total protein content was observed in the cultures after exposure to danazol. Finally, it is also possible that danazol exposure might cause internalization of GnRH receptors, thereby decreasing the total number of available cell surface receptors. Further studies are needed to examine these possibilities.

It can be concluded from these studies that in addition to direct inhibition of steroidogenesis in the gonads<sup>18</sup> and adrenals,<sup>19</sup> danazol also causes a decrease in GnRH responsiveness of the pituitary gland by decreasing the available GnRH receptors.

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# Prostaglandin secretion by adrenal tissue of human anencephalic fetuses

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Previously we reported that the human fetal adrenal gland secreted various prostaglandins and that prostaglandin secretion was inhibited by endogenously synthesized glucocorticoids. Furthermore, we reported that the neocortex secreted larger quantities of prostaglandins than did fetal zone tissue and that the pattern of secretion of prostaglandins of the two zones differed. In the present investigation the rate and pattern of prostaglandin secretion by adrenal tissue of anencephalic fetuses were assessed and compared to either whole or separated zones of human fetal adrenal tissue. The rate of prostaglandin secretion into the culture medium was determined by measuring prostaglandin  $E_2$  or prostaglandin  $F_{2\alpha}$  with use of specific radioimmunoassays in media collected at 24-hour intervals. The rate of prostaglandin  $E_2$  secretion by adrenal glands obtained from two anencephalic fetuses declined rapidly from  $1.71 \pm 0.65$  ng/mg<sup>-1</sup> of protein per 24 hours and  $0.82 \pm 0.46$  ng/mg<sup>-1</sup> of protein per 24 hours on day 1, respectively, to  $0.36 \pm 0.03$  and  $0.12 \pm 0.04$  ng/mg<sup>-1</sup> of protein per 24 hours by the fifth day in culture. The rate and pattern of prostaglandin  $F_{2\alpha}$  secretion by anencephalic tissue was similar to that of prostaglandin  $E_2$ . The pattern of prostaglandin secretion by anencephalic adrenal tissue was similar to that observed in neocortex tissue, but the rate of prostaglandin secretion was less. When the rates of prostaglandin secretion by anencephalic and neocortex adrenal tissue were compared to the rates of secretion of cortisol, an inverse relationship was observed. Finally, when whole human fetal adrenal glands or anencephalic tissues were incubated in the presence of adrenocorticotrophic hormone, dexamethasone, metyrapone, or SU 10603, the data obtained seemed to suggest that the rate of prostaglandin secretion was regulated in both tissues by endogenously synthesized glucocorticosteroids. In summary, the pattern of secretion of prostaglandins by the anencephalic adrenal gland was similar to that of neocortex tissue, and the rate of secretion of prostaglandins was inhibited by endogenously synthesized cortisol. (AM J OBSTET GYNECOL 1986;154:373-8.)

**Key words:** Prostaglandin secretion, human anencephalic adrenol, glucocorticosteroid action

In previous investigations we<sup>1</sup> and others<sup>2-4</sup> found that the human fetal adrenal gland both in vivo and in vitro secretes large quantities of steroids, principally dehydroepiandrosterone sulfate. During fetal life the human fetal adrenal gland undergoes rapid growth, and by 28 weeks of gestation the gland may attain the size of the fetal kidney.<sup>5</sup> The ratio of the adrenal gland to total body weight of a term fetus is 15 to 20 times that of an adult. This increase in growth is due principally to the increase in the size and number of adrenocortical cells making up the fetal zone, which then undergoes atrophy after birth.

In contrast, the adrenal glands of human anencephalics are markedly atrophic. Generally it is assumed that the failure of adrenal growth and the atrophy ex-

hibited by the anencephalic adrenal gland are due to the low levels of adrenocorticotrophic hormone in these fetuses.<sup>6,7</sup> Histologic studies of anencephalic adrenal glands have shown that they are small because of atrophy of the fetal zone cells, which are reduced in number as early as 15 weeks' gestation.<sup>8,9</sup> In the normal human fetal adrenal gland the outer rim of cells known as the neocortex secrete principally cortisol, which is secreted in relatively small quantities in vivo and in vitro compared to the amount of dehydroepiandrosterone sulfate secreted by the inner fetal zone.<sup>3</sup> In contrast the anencephalic adrenal gland, which contains functional neocortex cells, secretes cortisol in vitro at rates similar to those of neocortex cells obtained from normal abortuses but little or no dehydroepiandrosterone sulfate.<sup>10</sup>

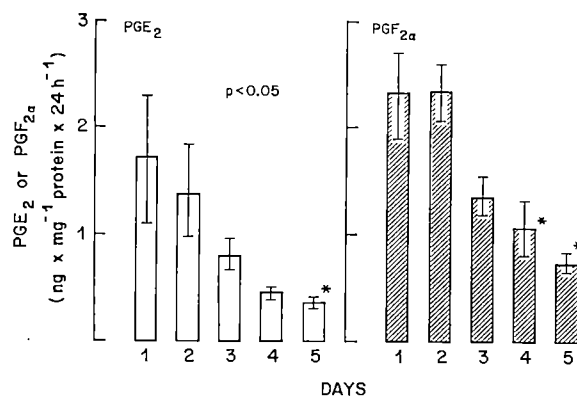
Previously we reported that the human fetal adrenal synthesizes and secretes a variety of prostaglandins and that prostaglandin secretion was regulated in part by endogenously synthesized glucocorticosteroids (cortisol).<sup>11</sup> In addition, we found that the neocortex secreted larger quantities of prostaglandins than did the fetal zone and that the pattern of secretion of prostaglandins between the two zones was different.<sup>12</sup> Furthermore,

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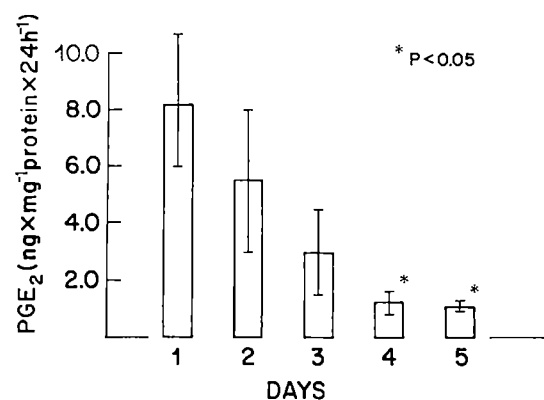


**Fig. 1.** The daily secretion rate of prostaglandin E<sub>2</sub> (open bar) and prostaglandin F<sub>2α</sub> (shaded bar) by adrenal tissue obtained from an anencephalic fetus of 34 weeks' gestation maintained in organ culture in the absence of added adrenocorticotrophic hormone. The medium was changed every 24 hours, and the concentration of prostaglandins was determined in the medium with use of radioimmunoassays. The results represent the mean and SE of values obtained from quadruplicate dishes (\* =  $p < 0.05$  compared to day 1).

prostaglandins may act in turn to modulate steroid secretion in human adrenal tissues.<sup>13</sup> The human anencephalic adrenal gland may provide an additional model for the study of adrenal prostaglandin secretion. The purpose of this present investigation was to determine the rate and pattern of secretion of prostaglandins by anencephalic adrenal tissues maintained in vitro compared to that of whole or separated fetal zone and neocortex human fetal adrenal tissues and to determine whether endogenously synthesized cortisol affects the rate of prostaglandin secretion.

#### Material and methods

**Adrenal glands.** Normal human fetal adrenal glands were obtained from first- and second-trimester abortions (10 to 22 weeks' gestation). Adrenal glands were also obtained from three anencephalic fetuses (34, 38, and 43 weeks' gestation) delivered vaginally after oxytocin-induced labor. Tissues were obtained in accordance with the Donors' Anatomical Gift Act of the State of Texas after consent was received in writing from the women to receive abortions or be delivered with use of a consent form and protocol approved by the Human Research Review Committee of the University of Texas Health Science Center at Dallas. Normal human fetal adrenal glands were obtained after abortion, which was accomplished by dilation and extraction. The neocortex was separated from the fetal zone by microdissection, as described previously.<sup>14</sup> The neocortex tissue that was attached to the capsule was estimated to be contaminated by  $\leq 10\%$  of the fetal zone tissue by histologic examination of tissue sections. The fetal zone was uniformly free of neocortex tissue. Pooled separated or

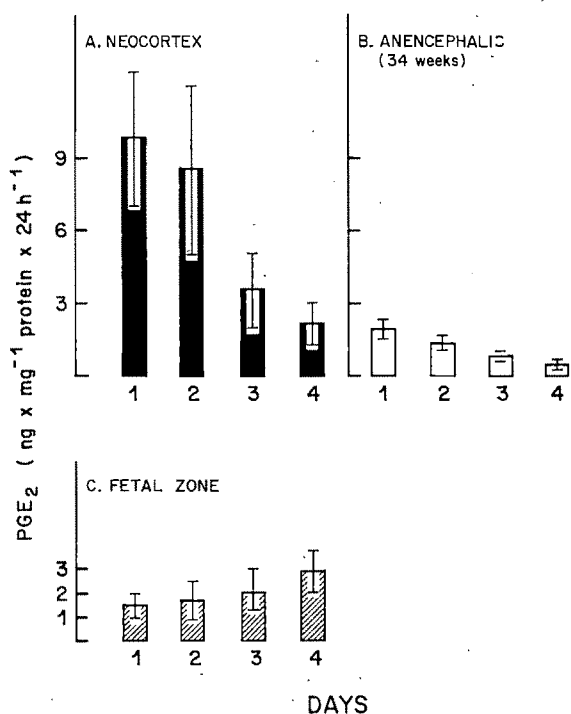


**Fig. 2.** The daily secretion rate of prostaglandin E<sub>2</sub> by adrenal tissue obtained from an anencephalic infant of 43 weeks' gestation maintained in the absence of added adrenocorticotrophic hormone. The medium was changed every 24 hours, and the concentration of prostaglandin E<sub>2</sub> was determined in the medium with use of radioimmunoassays. The results represent the mean and SE of values obtained from quadruplicate dishes (\* =  $p < 0.05$  compared to day 1).

whole mixed adrenal tissues were placed in Waymouth-Gey's culture medium containing 10% antibiotics and within 1 hour were minced into pieces of 1 mm<sup>3</sup>. The anencephalic adrenal glands were removed within 1 hour of delivery or death of the infant, and the peri-adrenal fat was removed and the tissue was minced as described for normal human fetal adrenal tissue.

**Tissue culture.** Three or four fragments of separated fetal zone, neocortex tissue, whole human fetal adrenal tissue, or anencephalic adrenal tissue (approximately 1 mg of tissue protein) were placed on lens paper supported by a stainless steel grid in each organ culture dish (60 × 15 mm; Falcon Plastics, Cockeysville, Maryland). The medium used consisted of 50 vol of Waymouth's MB 752/1 medium, 40 vol of Gey's Balanced Salt Solution (Grand Island Biological Company, Grand Island, New York), 1 vol of a 1% solution of antimycotics and antibiotics (Grand Island Biological), and 10 vol of whole human serum. The tissue fragments were maintained at 37° C in a humidified atmosphere of 5% carbon dioxide with 95% air. The medium was changed every 24 hours for 1 to 5 days, and the medium was stored at -20° C. In tissues maintained at 37° C, the rate of degradation of prostaglandin E<sub>2</sub> (assessed with use of tritiated prostaglandin E<sub>2</sub>) in the absence of tissue was  $< 10\%$  per day. At the end of the culture period the tissue fragments were rinsed thoroughly and homogenized in 0.5 ml of 0.15 mol/L of sodium chloride. Aliquots of the homogenates were taken for measurement of protein by the method described by Lowry et al.<sup>15</sup>

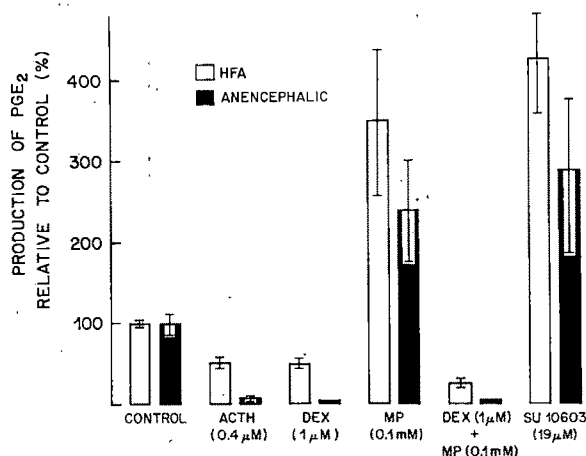
Each experimental condition was repeated in triplicate or quadruplicate, as indicated in the figure legends.



**Fig. 3.** A comparison of the daily rate of secretion of prostaglandin  $E_2$  by pooled separated neocortex (panel A) and fetal zone (panel C) tissue obtained from four adrenal glands from three abortuses and an anencephalic fetus of 34 weeks' gestation (redrawn from Fig. 1) (panel C) as a function of days in culture in tissues maintained in the absence of added adrenocorticotrophic hormone. The medium was changed daily and assayed for prostaglandin  $E_2$  concentration by radioimmunoassay. The results represent the mean and SE of values obtained from media of four replicate dishes (panels A and C) and quadruplicate dishes (panel B).

The results are expressed as nanograms of prostaglandins or micrograms of cortisol secreted into the culture medium per milligram of tissue per 24 hours (mean  $\pm$  SE). When indicated, synthetic adrenocorticotrophic hormone (1-24) (Cortrosyn) obtained from Organon (West Orange, New Jersey) was added to the culture medium to achieve a concentration of 0.4  $\mu\text{mol/L}$ . Dexamethasone obtained from Sigma Chemical Company (St. Louis, Missouri), metyrapone received from Aldrich (Milwaukee, Wisconsin), or SU 10603 [7-chloro-3,4-dihydro-2-(3-pyridyl)-naphthalen-1-one] provided by Ciba-Geigy (Summit, New Jersey) were added to the culture medium to achieve the concentrations indicated. Statistical analyses of the results obtained were performed with use of one-way analysis of variance and Newman-Keuls multiple comparison analyses.

**Measurement of prostaglandins.** Prostaglandins were measured by sensitive and specific radioimmunoassays, which had been fully described and validated previously.<sup>16</sup> The prostaglandins measured were prostaglandin  $E_2$  and prostaglandin  $F_{2\alpha}$ . The prostaglandin



**Fig. 4.** Effect of hormones and drugs on the production of prostaglandin  $E_2$  by mixed whole HFA tissue (open bars) (two adrenal glands from two abortuses) and by anencephalic adrenal tissue obtained from a 38-week-old fetus (solid bar). Fragments of tissue were incubated in medium containing whole human serum alone (control) or in medium containing whole human serum together with adrenocorticotrophic hormone (ACTH), 0.4  $\mu\text{mol/L}$ ; dexamethasone (DEX), 1  $\mu\text{mol/L}$ ; metyrapone (MP), 0.1 mmol/L; DEX (1  $\mu\text{M}$ ) plus MP, 0.1 mmol/L; or SU 10603, 19  $\mu\text{mol/L}$ , as indicated. The media was changed every 24 hours, and prostaglandin  $E_2$  was assayed in the medium collected on the fourth day of culture. Results represent mean and SE of values obtained from media of four replicate dishes and are normalized to control equals 100%.

$F_{2\alpha}$  antiserum was a gift of Dr. K. T. Kirton (see end of text) and came from the same batch as that used previously.<sup>16</sup> The prostaglandin  $E_2$  antiserum was produced by one of us and has been described in detail elsewhere.<sup>17</sup> The lower limits of sensitivity of the assays were in the range of 1 to 5 pg per tube, and the intraassay coefficients of variation were  $<10\%$ . The cross-reactivities of the antisera with other prostaglandins measured were  $<1\%$ .

The contribution of prostaglandins in the culture medium prepared, that is, in the human serum (approximately 2 ng/ml for each prostaglandin), was corrected for by exposure of the authentic prostaglandin standards in the standard curve to the appropriate matrix. Hence the rates of formation quoted are already corrected for endogenous prostaglandin content of the culture medium.

**Measurement of cortisol.** Cortisol secretion was determined by radioimmunoassay as described by Mason et al.<sup>18</sup> Results were presented as micrograms of cortisol secreted per milligram of tissue protein per 24 hours.

## Results

The daily secretion rates of prostaglandin  $E_2$  and prostaglandin  $F_{2\alpha}$  in the absence of adrenocorticotrophic hormone by adrenal tissue from anencephalic fetus 1 (34 weeks' gestation) is presented in Fig. 1. Daily se-



**Table 1.** Daily secretion rate of cortisol (micrograms per milligram of protein per 24 hours) by anencephalic adrenal tissue (mean  $\pm$  SE)

	Days				
	1	2	3	4	5
Anencephalic fetus 1 (34 weeks)	0.23 $\pm$ 0.03	0.53 $\pm$ 0.07	0.89 $\pm$ 0.18*	1.12 $\pm$ 0.15*	1.07 $\pm$ 0.14*
Anencephalic fetus 2 (43 weeks)	1.32 $\pm$ 0.25	3.52 $\pm$ 0.44*	2.63 $\pm$ 0.91*	2.94 $\pm$ 0.32*	2.31 $\pm$ 0.85*

\* $p < 0.05$  (compared to day 1).

cretion rates of prostaglandin  $E_2$  and  $F_{2\alpha}$  declined rapidly from  $1.71 \pm 0.65$  and  $2.32 \pm 0.41$  ng/mg of protein per 24 hours (mean  $\pm$  SE) to  $0.36 \pm 0.03$  and  $0.67 \pm 0.09$  ng/mg of protein per 24 hours by day 5 in culture ( $p < 0.05$ ). Since the secretory rates for prostaglandin  $E_2$  and  $F_{2\alpha}$  were similar, in further experiments prostaglandin  $E_2$  alone was measured.

A similar pattern of secretion was observed for prostaglandin  $E_2$  by adrenal tissue from anencephalic fetus 2 (43 weeks' gestation) (Fig. 2). The daily secretion rate fell from  $0.82 \pm 0.46$  on day 1 to  $0.12 \pm 0.04$  ng/mg of protein per 24 hours by day 5 in culture ( $p < 0.05$ ).

A comparison of the daily rate of secretion of prostaglandin  $E_2$  in separated fetal zone, neocortex, and adrenal tissue from anencephalic fetus 1 is illustrated in Fig. 3. The rate of secretion of prostaglandin  $E_2$  by anencephalic adrenal tissue was lower than that observed in neocortex but similar to that observed by fetal zone tissue. However, the pattern of secretion by anencephalic adrenal tissue was similar to that observed in neocortex tissue.

To determine whether the rate of secretion of steroids and in particular cortisol was related to prostaglandin secretion, we measured the daily secretion rate of cortisol into the culture medium (Table 1). The rate of cortisol secretion appeared to be low on day 1 in both anencephalic tissues but increased and plateaued by day 3 in anencephalic fetus 1 and by day 2 in anencephalic fetus 2. The secretion of dehydroepiandrosterone sulfate was barely detectable in both experiments (data not shown).

We previously provided evidence that endogenously produced glucocorticosteroids (most likely cortisol) inhibit prostaglandin biosynthesis in normal whole human fetal adrenal<sup>11</sup> and in separated zones of human fetal adrenal tissue.<sup>12</sup> To determine whether similar mechanisms occurred in anencephalic adrenal tissue we investigated the effects of adrenocorticotrophic hormone, dexamethasone, SU 10603 (an inhibitor of  $17\alpha$ -hydroxylase activity), and metyrapone (an inhibitor of  $11\beta$ -hydroxylase activity) on normal whole human fetal adrenal tissue and on anencephalic adrenal tissue (anencephalic fetus 3, 38 weeks' gestation); the results

are presented in Fig. 4. Inhibition of prostaglandin secretion in both normal and anencephalic adrenal tissue occurred in tissues incubated for 4 days in the presence of adrenocorticotrophic hormone and dexamethasone. Inhibition of cortisol biosynthesis by metyrapone and SU 10603 resulted in a threefold to fourfold increase in the rate of prostaglandin secretion. The addition of dexamethasone to metyrapone inhibited the stimulation of prostaglandin synthesis observed in tissue incubated in the presence of metyrapone alone.

#### Comment

In previous investigations we reported that both whole human fetal adrenal tissue and separated neocortex and fetal zone tissue maintained in tissue culture synthesize and secrete a variety of prostaglandins and thromboxane  $B_2$ ,<sup>11, 12</sup> and the secretion of prostaglandins and thromboxane  $B_2$  is regulated by glucocorticosteroids that are produced in response to adrenocorticotrophic hormone.

Since anencephalic and neocortex tissue are exposed in vivo to different hormonal environments, that is, high estrogen and high adrenocorticotrophic hormone in normal neocortex tissue and low estrogen and low adrenocorticotrophic hormone in anencephalic tissue, we had proposed that the pattern of prostaglandin secretion might be different between the two types of tissue. Furthermore, we believed that since anencephalic and neocortex tissue are not exactly similar with respect to histology, steroid secretory pattern, expression of adenylate cyclase, and steroid hydroxylases, such factors may also affect the pattern of prostaglandin secretion in anencephalic and neocortex tissues.<sup>10, 13, 19, 20</sup> We observed, however, that the pattern of secretion by anencephalic adrenal tissue (that is, a decline) was similar to that observed for neocortex tissue, probably because anencephalic adrenal tissue is largely composed of neocortex tissue. These results suggest that the observed differences between anencephalic and neocortex tissues did not in fact affect the pattern of prostaglandin secretion between the two zones. The observed difference in the rate of prostaglandin secretion between neocortex and anencephalic adrenal tissue might

be related to differences in fetal age, functioning fetal zone tissue, rates of glucocorticoid biosynthesis, or different hormonal milieu.

In contrast, the rate and pattern of prostaglandin secretion in fetal zone tissue increased as a function of time in culture as reported previously.<sup>12</sup> It is possible that prostaglandins synthesized *in situ* might act as modulators of steroid secretion by the human fetal adrenal gland and the endogenously synthesized prostaglandins may bring about necrosis of the fetal zone, which is known to occur within the first few weeks of postnatal life,<sup>21</sup> either directly or by influencing blood flow.

Previously we were unable to demonstrate significant differences in the rate of cortisol secretion in the culture medium between neocortex and fetal zone tissue and were unable to explain differences in the rate and pattern of secretion of prostaglandins between the two zones.<sup>12,14</sup> In the present experiment we observed that the rate of cortisol secretion of anencephalic adrenal glands, in fact, increased approximately twofold as a function of days in culture.

From these results it appears that the rate of secretion of prostaglandins by anencephalic tissue is high on the first day in culture at the same time that cortisol secretion is low. With progressive days in culture, prostaglandin secretion falls and cortisol secretion rises and plateaus by 2 to 3 days. The reason for a small but significant rise in cortisol secretion is not completely clear. A possible explanation for these results might be that prostaglandins suppress cortisol secretion (as demonstrated previously *in vitro*).<sup>13</sup> Another possible explanation could be that there were small amounts of adrenocorticotrophic hormone in the human serum added to the culture medium sufficient to stimulate cortisol secretion by anencephalic but not normal human fetal adrenal tissue. It is unclear whether the fall or the rise of prostaglandin secretion occurs primarily or secondarily to changes in cortisol secretion. We have also demonstrated that endogenously secreted glucocorticosteroids inhibit prostaglandin secretion and that prostaglandin secretion increases when cortisol biosynthesis is inhibited in both normal human fetal adrenal tissue as reported previously<sup>11</sup> and in anencephalic adrenal tissue (Fig. 4).

In conclusion, the pattern of prostaglandin secretion by anencephalic adrenal tissue is similar, but the rate of secretion is less than that observed by neocortex tissue. Furthermore, there appears to be an inverse relationship between endogenously formed glucocorticosteroids and prostaglandin secretion in anencephalic adrenal tissue.

We are grateful to Dr. K. T. Kirton (Upjohn Company, Kalamazoo, Michigan) for the gift of prostaglan-

din F<sub>2a</sub> antiserum. We would like to thank Nora Cline for her expert technical assistance and Lynne McDonnell for her editorial assistance in the preparation of this manuscript.

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## Pulmonary responses to exercise in pregnancy

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The pulmonary responses of 88 pregnant women were compared to those of 39 nonpregnant control subjects during different exercise intensities. At rest the pregnant women had higher tidal volumes, oxygen consumption, carbon dioxide production, and respiratory exchange ratio. With increased work loads the pregnant volunteers have consistently lagged behind the nonpregnant control subjects for every parameter, which indicates a decrease in pulmonary reserve and inability to exercise anaerobically. (AM J OBSTET GYNECOL 1986;154:378-83.)

**Key words:** Exercise in pregnancy, pulmonary physiology

The physiologic requirements and normal changes of pregnancy include close interaction between cardiovascular and respiratory functions. The mechanism by which oxygen and carbon dioxide are transported between the atmosphere and the cells of the mother and fetus is quite complex.

As pregnancy progresses, individuals who exercise may experience increasing intolerance to exercise, which is caused by the inability to transfer gas (oxygen and carbon dioxide) between the atmosphere to cells; such deficiencies are usually compensated in nonpregnant individuals by increased pulmonary diffusing capacity and increased alveolar ventilation. During pregnancy, hemoglobin, oxygen-carrying capacity, and cardiac output increase significantly and in excess of demands, which leads to decreased arteriovenous oxygen difference.

In this study we report a comparison between pulmonary responses of pregnant and nonpregnant individuals to mild, moderate, and maximal oxygen consumption single-session exercise.

### Material and methods

This study was approved by the Institutional Review Board, and informed consent forms were obtained from all subjects. The report includes preliminary data

derived from a larger ongoing study. A total of 127 healthy subjects were entered into the study; 88 were pregnant and 39 were not. Table I indicates the number of patients entered in each exercise group.

Table II summarizes a few characteristics of the subjects obtained at the time they entered the study.

Based on an intake questionnaire, the individuals in both groups were found to be of similar levels of fitness as judged by their regular physical activities. The data indicated that both groups had been involved at an average level in regular organized physical activities. We expected the most significant differences to emerge in the second half of pregnancy, so the pregnant subjects were tested either in the late second or third trimester of their pregnancy at a mean gestational age of  $28.8 \pm 1.6$  weeks. The complexity of the study precluded serial evaluations of the subjects.

We used similar protocols in all our studies—only the work load was different. The subjects were randomly assigned to the different exercise groups. Before exercise all subjects were placed in a semisupine position for a control period of 30 minutes. Then the subjects exercised; the mild exercise consisted of a 15-minute walk on a motorized treadmill at a constant speed of 2 mph (energy utilization of approximately 2.3 to 3.0 MET). The moderate exercise also included a 15-minute walk at a speed of 2 mph, but this time the treadmill gradient was elevated to a 10% inclination (energy utilization of approximately 5 to 6 MET). The strenuous exercise included a symptom-limited maximal oxygen consumption test during which the speed of the treadmill was maintained at 2.5 mph and the treadmill gradient was elevated by 2% every minute until the subjects

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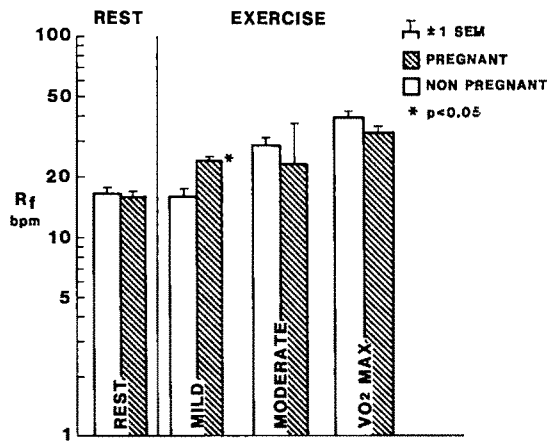


Fig. 1. Respiratory frequency ( $R_f$ ) in breaths per minute (bpm) as measured during rest and at the peak of mild, moderate, and maximal oxygen consumption ( $VO_2 \max$ ) exercise. The asterisk indicates statistical difference between pregnant and control subjects at that particular work load.

reached exhaustion (energy utilization of approximately 7 to 9 MET). The exercise was followed by a 30-minute recovery period, again in the semisupine position. Several variables were monitored throughout the experiments: maternal heart rate, maternal arterial blood pressure, and fetal heart rate. Whenever technically feasible, the fetal heart rates were also recorded during exercise with either a Hewlett-Packard 8040-A or a Corometrics 210 cardiotocograph). During exercise the subjects breathed into an open-circuit continuous gas sampling system. Mixed expired oxygen and carbon dioxide were analyzed with a Beckman metabolic cart. The exercise was followed by a 30-minute recovery period, again in the semisupine position.

Statistical analysis was done by paired  $t$  test, two-sample  $t$  tests, and analysis of variance as indicated.

## Results

The results of our study are illustrated in Figs. 1 to 7. Results are expressed as mean  $\pm 1$  SEM.

**Respiratory frequency.** Fig. 1 illustrates the respiratory frequency in breaths per minute of pregnant subjects compared to that of nonpregnant control subjects at rest and during mild, moderate, and maximal oxygen consumption exercise ( $VO_2 \max$ ).

At rest the respiratory frequency was comparable in both groups,  $16.6 \pm 1.23$  bpm in the pregnant subjects and  $16.1 \pm 0.9$  bpm in the control subjects. During mild exercise the respiratory frequency in the pregnant subjects increased significantly ( $p < 0.05$ ) to  $24.4 \pm 1.1$  bpm in comparison to that of the control subjects,  $16.0 \pm 2.7$  bpm. With moderate and maximal oxygen consumption exercise the pregnant subjects increased their respiratory frequency to  $26.0 \pm 3.7$  bpm and to  $32.92 \pm 1.3$  bpm, respectively, and the control subjects

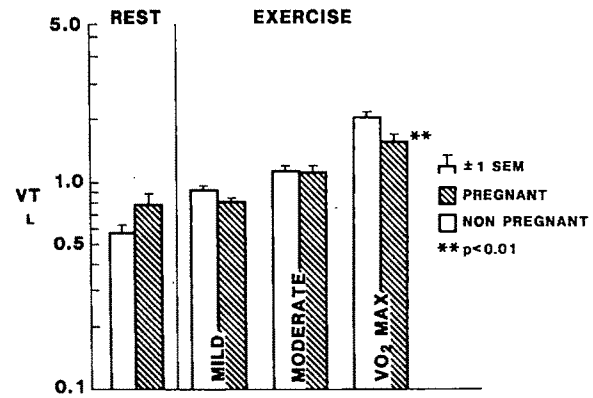


Fig. 2. Tidal volume ( $VT$ ) in liters (L) during rest and at the peak of mild, moderate, and maximal oxygen consumption exercise.

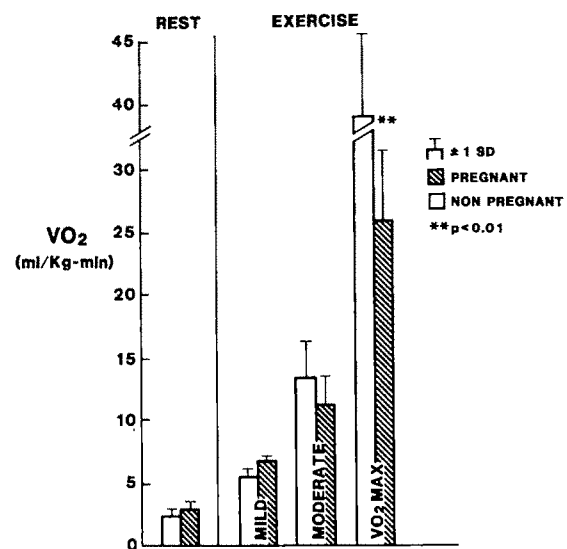


Fig. 3. Oxygen consumption ( $VO_2$ ) determinations obtained at rest and then at the peak of mild, moderate, and maximal oxygen consumption exercise.

to  $28.3 \pm 1.8$  bpm and  $38.4 \pm 2.3$  bpm, respectively.

**Tidal volume.** At rest the tidal volume tended to be consistently elevated in all pregnant subjects,  $0.78 \pm 0.8$  L, when compared with that of the nonpregnant control subjects,  $0.58 \pm 0.04$  L (Fig. 2). With increased work load the pregnant women had lower proportional increases in comparison with the nonpregnant women. At maximal oxygen consumption exertion the tidal volume was significantly ( $p < 0.01$ ) lower in the pregnant subjects,  $1.73 \pm 0.09$  L, than in the nonpregnant control subjects,  $2.13 \pm 0.2$  L.

**Oxygen consumption.** Fig. 3 illustrates the oxygen consumption determinations. At rest the maximal oxygen consumption was higher in the pregnant subjects at  $3.0 \pm 0.9$  ml/kg/min ( $0.365 \pm 0.06$  L) than in the nonpregnant control subjects,  $2.5 \pm 0.9$  ml/kg/min ( $0.23 \pm 0.02$  L). With mild exercise oxygen consump-



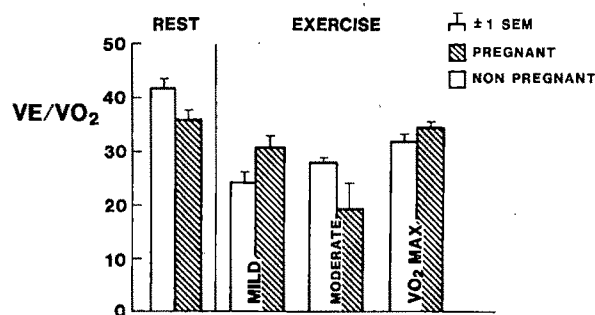


Fig. 4. Ventilatory equivalent ( $VE/VO_2$ ) during rest and at the peak of mild, moderate, and maximal oxygen consumption exercise.

tion reached slightly higher levels in the pregnant subjects,  $8.0 \pm \text{ml/kg/min}$  ( $0.68 \pm 0.01 \text{ L}$ ), whereas in the nonpregnant control subjects the increment was to  $6.0 \pm 1.0 \text{ ml/kg/min}$  ( $0.61 \pm 0.02 \text{ L}$ ). With moderate and maximal oxygen consumption exercise the oxygen consumption increase lagged in the pregnant subjects,  $12.0 \pm 3 \text{ ml/kg/min}$  ( $1.25 \pm 0.04 \text{ L}$ ) and  $25 \pm 5 \text{ ml/kg/min}$  ( $1.79 \pm 0.09 \text{ L}$ ), respectively, in comparison to  $14.0 \pm 2.5 \text{ ml/kg/min}$  ( $1.35 \pm 0.08 \text{ L}$ ) and  $38.0 \pm 7.0 \text{ ml/kg/min}$  ( $2.04 \pm 0.2 \text{ L}$ ) in the control subjects.

**Ventilatory equivalent.** As seen in Fig. 4, the ventilatory equivalent/oxygen consumption at rest was  $36 \pm 1.8$  in the pregnant subjects and  $42.7 \pm 2.6$  in the nonpregnant control subjects. With mild exercise the ventilatory equivalent/oxygen consumption decreased in the pregnant subjects to  $32.2 \pm 1$  and in the control women to  $23.9 \pm 2.3$ . It is of interest that during the moderate exercise period we observed a further decrease in ventilatory equivalent/oxygen consumption in the pregnant women to  $19.2 \pm 4.6$  but a slight increase in the control subjects to  $28.8 \pm 0.9$ . Maximal oxygen consumption exercise induced a significant increase in the ventilatory equivalent/oxygen consumption ratio in both pregnant women to  $34.7 \pm 1.0$  and nonpregnant subjects to  $31.4 \pm 1.28$ . Ventilatory equivalent/oxygen consumption did not appear to be statistically different in the pregnant as compared to nonpregnant subjects, but overall the ventilatory equivalent/oxygen consumption tended to be slightly lower in the control subjects. This difference can be better illustrated when ventilatory equivalent/oxygen consumption is plotted for the same individuals during maximal oxygen consumption exercise at 3 minutes before exercise and then at 1-minute intervals into a symptom-limited maximal oxygen consumption exercise (Fig. 5).

**Carbon dioxide production.** As shown in Fig. 6, the resting carbon dioxide production before exercise was  $0.27 \pm 0.02 \text{ L/min}$  in the pregnant subjects and  $0.18 \pm 0.02 \text{ L/min}$  in the control subjects. With mild exercise carbon dioxide production increased in the pregnant women,  $0.53 \pm 0.02 \text{ L/min}$ , significantly

more ( $p < 0.05$ ) than in the control subjects,  $0.43 \pm 0.02 \text{ L/min}$ .

At the peak of moderate exercise the carbon dioxide production increased similarly in both pregnant women,  $0.96 \pm 0.02 \text{ L/min}$ , and nonpregnant control subjects,  $1.14 \pm 0.8 \text{ L/min}$ . During maximal oxygen consumption exercise the pregnant subjects maintained a significantly lower ( $p < 0.01$ ) carbon dioxide production of  $1.98 \pm 0.1 \text{ L/min}$  in comparison to  $2.73 \pm 0.3 \text{ L/min}$  in the control women.

**Respiratory quotient (respiratory exchange ratio).** Fig. 7 shows that during mild exercise the respiratory quotient was  $0.71 \pm 0.08$  in the control subjects and  $0.78 \pm 0.07$  in the pregnant volunteers. At the peak of moderate exercise the respiratory quotient was similar in both groups— $0.84 \pm 0.1$  in the pregnant and control subjects. At the peak of maximal oxygen consumption exercise the respiratory quotient changed to  $1.1 \pm 0.02$  in the pregnant and  $1.3 \pm 0.02$  in the nonpregnant control subjects.

**Fetal heart rate.** By and large, we have observed a significant increase in fetal heart rate baseline after exercise (Artal R, Romen Y, Wiswell RA. Fetal heart rate responses to maternal exercise, unpublished observations), and in three cases we have previously reported fetal bradycardia during exercise.<sup>1</sup> No correlation was found between fetal heart rate and the maternal pulmonary responses to exercise.

### Comment

The purpose of this study was to review the adaptive changes of the respiratory system during exercise in pregnancy.

Pregnancy is characterized by various anatomic changes, and the most significant are summarized below. The upper respiratory tract is often affected by changes in the mucosa of the nasopharynx such as hyperemia, edema, and excessive secretion, all causing obstructive symptoms. The rib cage undergoes changes in pregnancy which result in an expansion of the chest circumference resulting from the elevation of the diaphragm by the growing uterus.<sup>2-5</sup> These changes are more prominent during the second half of pregnancy and lead to an increase in inspiratory capacity of 300 ml (tidal volume plus inspiratory volume) and a reduction in functional residual capacity<sup>6,7</sup> to maintain intact total lung and vital capacity. Significantly, pregnancy at rest is characterized by a 10% to 20% increase in oxygen consumption. The combination of reduced functional residual capacity and increased oxygen consumption results in lower oxygen reserve. If not properly compensated, the oxygen reserve could be further lowered during heavy exercise and potentially lead to hypoxia. The resting minute ventilation is increased by 40% to 50%, leading to a decrease of arterial  $PCO_2$  to

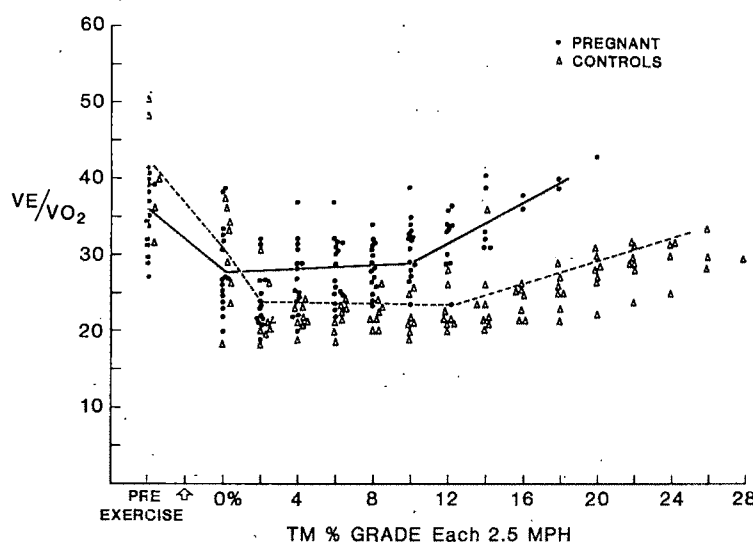


Fig. 5. Ventilatory equivalent ( $VE/VO_2$ ) determinations done before and during symptom-limited maximal oxygen consumption exercise. The *solid line* illustrates the mean values for the pregnant subjects and the *broken line* the nonpregnant control subjects.

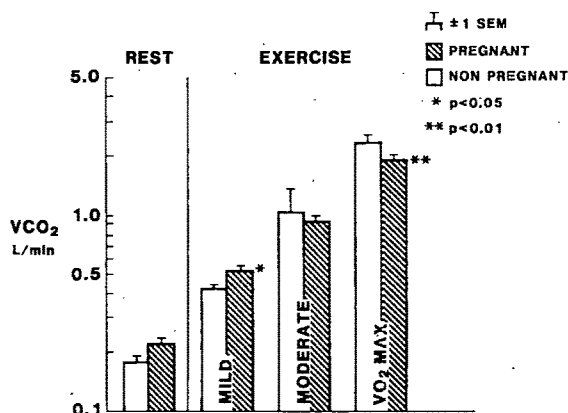


Fig. 6. Carbon dioxide production ( $VCO_2$ ) at rest and as determined at the peak of mild, moderate, and maximal oxygen consumption exercise.

30 mm Hg and an arterial  $PO_2$  of approximately 105 mm Hg. The respiratory alkalosis is due to the effect of estrogen and progesterone on the respiratory center of the brain. Acid base status is maintained by a compensatory metabolic acidosis. This results in a decrease in serum bicarbonate of approximately 20 mEq/L. The arterial pH is approximately 7.44.

Arterial  $PO_2$  can be significantly reduced in pregnancy in the supine position. In supine position, hypoventilation of a lesser degree, or hyperventilation leading to systemic maternal alkalosis in pregnancy can significantly increase the risk of hypoxia in the fetus.

Functionally, the pregnant woman compensates for all of the above changes by breathing more deeply.

In general, exercise studies conducted or performed on pregnant women lack controls and standardization,

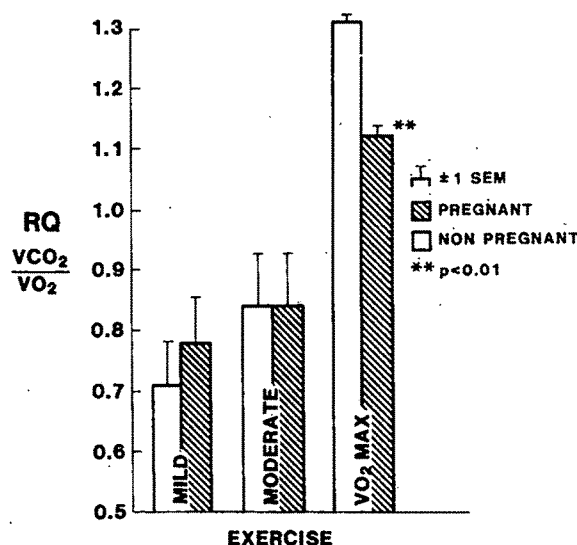


Fig. 7. Respiratory quotient (RQ), or respiratory exchange ratio, at the peak of mild, moderate, and maximal oxygen consumption exercise.

ignore state of fitness, or extrapolate it from estimated maximal oxygen consumption data. Only a few studies carried their subjects to maximal oxygen consumption. Very little attention has been paid to distinguishing between weight-bearing and non-weight-bearing exercise.

Weight-bearing exercises are performed less efficiently and are more energy costly in pregnancy, since they contain a component of body weight. Few studies have been published on pulmonary responses to exercise in pregnancy and after delivery.<sup>7-15</sup> Since the methods, intensity of exercise, and level of fitness in

**Table I.** Subjects per protocol

Subject	Mild exercise	Moderate exercise	VO <sub>2</sub> max exercise
Pregnant	43	20	25
Nonpregnant	15	14	10

**Table II.** Characteristics of subjects (mean  $\pm$  1 SD)

	Nonpregnant	Pregnant
Age (yr)	30.4 $\pm$ 6.1	28.2 $\pm$ 5.5
Weight (pounds)	140 $\pm$ 43	148 $\pm$ 40
Resting mean arterial blood pressure (mm Hg)	83 $\pm$ 8.5	84 $\pm$ 8.7
Resting heart rate (bpm)	67.9 $\pm$ 9.0	81.2 $\pm$ 9.00

**Table III.** Comparison of pulmonary responses to exercise in pregnant and control subjects

Author of study	No. of patients	Respiratory frequency	Minute ventilation	Oxygen consumption	Tidal volume	Ventilatory equivalent	Respiratory exchange ratio	Exercise	Intensity
Bader <sup>8</sup>	46			↑				Ergometer	Steady rate
Guzman <sup>9</sup>	8	↑/NC	↑	NC	NC			Ergometer	150 kpm*
Ueland <sup>10</sup>	22			↑				Ergometer	100 kpm
Knuttgen <sup>7</sup>	13	NC	↑	↑	↑	↑	↑	Treadmill	380 kpm
Knuttgen <sup>7</sup>	13	NC	↑	NC	↑	↑	NC	Ergometer	367 kpm
Pernoll <sup>11</sup>	12	↑/NC	↑	↑	↑		NC	Ergometer	306 kpm
Collings <sup>12</sup>	20			↑				Ergometer	Submaximal
Artal (present study)	58	↑	↑/NC	NC	NC	↑	↑/NC	Treadmill	210 kpm
Artal (present study)	34	↑	↑/NC	NC	NC	↑	↑/NC	Treadmill, 10% grade	350 kpm
Artal (present study)	35	↓	↓	↓	↓	↑	↓	Treadmill	Maximal oxygen uptake, 650 kpm

↑ = Increase; NC = no change; ↓ = decrease.

\*One hundred kilopond meters (kpm) = 16.35 watts.

the different studies cannot be compared in absolute values, we have summarized the data published by their relative changes (Table III).

From the above studies it appears that respiratory frequency tends to increase with either weight-bearing or non-weight-bearing exercises. Such an increase is quite similar to that occurring in nonpregnant controls. Most of the studies have found a significant increase in minute ventilation and tidal volume, not only at rest but also during and following exercise. The changes were significantly higher for the degree of exercise than in nonpregnant control subjects. The disproportionate increase in minute ventilation as compared to oxygen consumption is reflected in a relatively high ventilatory equivalent for oxygen.

The testing of maximal oxygen consumption has been traditionally conducted in weight-bearing exercise; so we have selected this same testing modality. We are cognizant that such testing may have significant limitations in pregnancy because of the weight changes that occur in pregnancy. Nevertheless, this type of activity reproduces more than any other the real daily life activities. To account for weight differences, our data were expressed also in milliliters per kilogram. Weight may be a factor during mild and moderate exercise but not during maximal oxygen consumption

exercise, which very accurately reflects exercise and physical work capacity.

In our present studies we compared the pulmonary responses to mild, moderate, and maximal oxygen consumption exercise. During mild exercise the respiratory frequency of pregnant women was found to be significantly higher than that of control subjects (Fig. 1). During the same type of exercise the minute ventilation appeared to be slightly increased in pregnancy whereas oxygen consumption and tidal volume were found to be comparable for the same amount of work (Figs. 2 and 3). The relative increase in minute ventilation resulted in a higher ventilatory equivalent during this type of exercise in pregnancy as compared to that in control subjects (Fig. 4). During maximal oxygen consumption exercise the pregnant women were not capable of matching the responses of the nonpregnant control subjects.

On the whole, the pregnant subjects responded to exercise with increased ventilation at mild and maximal exercise.

It appears that, contrary to our predictions, pregnant subjects demonstrate a more efficient ventilatory response to moderate exercise (5 to 6 MET) when compared to nonpregnant control subjects as demonstrated by a trend toward lower ventilatory equivalent. It can

be hypothesized that the improved response during moderate exercise is a result of the primary respiratory alkalosis of pregnancy.

Tidal volumes were similar during mild and moderate exercise between the groups. At maximal oxygen consumption exercise the primary respiratory alkalosis is not sufficient to compensate for the developing metabolic acidosis. Further support for the above findings is the fact that carbon dioxide production during moderate exercise is slightly lower because of a favorable respiratory equivalent (Fig. 5).

It has been recognized for a long time that maternal hyperventilation, when producing respiratory alkalosis in the mother, may lead to fetal respiratory alkalosis.<sup>14</sup> The fall in maternal and fetal  $PCO_2$  is associated with a corresponding fall in fetal  $PO_2$ .

Our data indicate that during intensive exercise, pregnant women use proportionately less carbohydrates as their fuel source and fat becomes the principal source of energy, as reflected in lower respiratory quotient during intensive exercise coupled by increased ventilation and respiratory acidosis. This result may indicate an inability to exercise anaerobically, which may be a protective mechanism from hypoxia or may reflect a protective mechanism to maintain steady levels of carbohydrates. In other words, the lower maximal oxygen consumption may be a result of decreased fuel and may also reflect the inability of the mother to allow a state of hypoxia or hypoglycemia (Fig. 7).

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# Hyperinsulinemia in hyperthecosis of the ovaries

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Fasting insulin concentrations and the insulin response to an oral glucose tolerance test were measured in six virilized women with ovarian hyperthecosis and six weight-matched normal women. For comparison, six women with polycystic ovarian disease were also studied. The diagnosis of hyperthecosis was confirmed in all six virilized women by histologic examination of the ovaries. The fasting insulin concentrations were increased in all of the hyperthecosis patients ( $84 \pm 32 \mu\text{U/ml}$ ). Insulin response to an oral glucose tolerance test was greatly increased ( $p < 0.01$ ) in comparison to normal women and women with polycystic ovarian disease. Significant positive correlations were found between peripheral insulin concentrations and ovarian vein testosterone ( $r = 0.879$ ,  $p < 0.02$ ), dihydrotestosterone ( $r = 0.866$ ,  $p < 0.03$ ), and androstenedione ( $r = 0.992$ ,  $p < 0.01$ ) levels. Insulin resistance persisted after removal of the ovaries even though androgen levels returned to normal. These results suggest that a significant degree of insulin resistance exists in women with hyperthecosis and that insulin stimulates ovarian stromal androgen synthesis and thus may play a role in the pathogenesis of ovarian hyperthecosis. (AM J OBSTET GYNECOL 1986;154:384-9.)

**Key words:** Insulin resistance, hyperthecosis, stromal luteinization

Hyperthecosis is a syndrome characterized by presence of large nests of luteinized cells in the ovarian stroma associated clinically by masculinization.<sup>1</sup> Patients with hyperthecosis are not only severely hirsute but also virilized. Unlike polycystic ovarian disease, in which the luteinized thecal cells are confined to areas around the cystic follicles, in hyperthecosis large islands of luteinized cells are scattered all over the stroma away from the follicles.<sup>2</sup> The cause of hyperthecosis or diffuse luteinization of the stroma of the ovaries is unknown. Women with hyperthecosis do not have the tonic elevation of LH as seen in polycystic ovarian disease and their luteinizing hormone response to luteinizing hormone-releasing hormone is in the normal range.<sup>3,4</sup> Hence, in addition to luteinizing hormone, there should be other factors stimulating ovarian stromal androgen production in these women.

In vitro incubation studies indicate that insulin stimulates porcine thecal steroidogenesis.<sup>5</sup> The present study was undertaken to investigate whether women with hyperthecosis have insulin resistance and an increase in immunoreactive insulin levels, whether insulin stimulates ovarian stromal androgen synthesis, and

whether the insulin resistance is secondary to hyperandrogenism.

## Material and methods

Six patients with a long-standing history of hirsutism and virilization were included in the study. Hirsutism was so severe that all had to shave daily. All were nulliparous; they had failed to ovulate with administration of Clomid, and their hirsutism did not improve with administration of oral contraceptives. All had signs of virilization: clitoral enlargement, temporal balding, and masculine body habitus. None had clinical features of acanthosis nigricans. All were obese ( $\geq 20\%$  above their ideal body weight (Metropolitan Life Insurance Company tables). Their follicle-stimulating hormone and luteinizing hormone levels were low or normal when determinations were done at two or more occasions. Prolactin levels were in the normal range in all patients ( $< 20 \text{ ng/ml}$ ). For comparison, six women with polycystic ovarian disease who matched the hyperthecosis patients in percentage of ideal body weights were also studied. These women were hirsute with no virilization and had anovulatory cycles and had elevated luteinizing hormone levels, with a luteinizing hormone/follicle-stimulating hormone ratio of  $> 3$ . Six nonhirsute women with normal ovulatory cycles who matched the patients in body weight served as control subjects (Table I).

After a high-carbohydrate (300 gm) diet was observed for 3 days, a standard oral glucose tolerance test was performed. Blood samples for glucose and insulin

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**Table I.** Clinical data for patients and controls

Patient	Percentage of ideal body weight*	Hirsutism	Virilization	Menstrual history	Follicle-stimulating hormone†	Luteinizing hormone‡
With hyperthecosis						
1	149	Severe	Yes	Amenorrhea	9	13
2	167	Severe	Yes	Oligomenorrhea	8	7
3	157	Severe	Yes	Amenorrhea	15	22
4	127	Severe	Yes	Oligomenorrhea	12	4
5	174	Severe	Yes	Oligomenorrhea	6	5
6	199	Severe	Yes	Amenorrhea	14	4
Mean ± SE	162 ± 10					
With polycystic ovarian disease						
1	141	Yes	No	Oligomenorrhea	9	42
2	170	Yes	No	Oligomenorrhea	7	60
3	152	Yes	No	Amenorrhea	11	54
4	122	Yes	No	Oligomenorrhea	11	67
5	171	Yes	No	Oligomenorrhea	8	31
6	196	Yes	No	Amenorrhea	12	58
Mean ± SE	159 ± 11					
Control						
1	146	No	No	Ovulatory	—	—
2	160	No	No	Ovulatory	—	—
3	152	No	No	Ovulatory	—	—
4	122	No	No	Ovulatory	—	—
5	174	No	No	Ovulatory	—	—
6	158	No	No	Ovulatory	—	—
Mean ± SE	152 ± 7					

Actual body weight

\* Ideal body weight × 100.

†Normal range: 5-15 mIU/ml.

‡Normal range: 5-25 mIU/ml.

determinations were obtained before oral glucose administration and every hour for 3 hours afterwards. All six virilized patients subsequently underwent laparotomy. Hysterectomy with bilateral salpingo-oophorectomy was performed in all of the hyperthecosis patients to treat the severe progressive hirsutism and virilization that had not responded to ovarian suppression with oral contraceptives. Simultaneous blood samples were obtained from the ovarian veins and peripheral veins at the time of operation. An oral glucose tolerance test with determination of insulin levels was repeated 3 months after operation. Postoophorectomy levels of all the androgens were also measured at this time. The ovaries of all the patients were examined histologically.

Insulin levels were measured by the previously described radioimmunoassay procedure.<sup>7</sup> Levels of testosterone, androstenedione, and dihydrotestosterone were measured by radioimmunoassay after fractionation by celite microcolumn chromatography as previously described.<sup>8</sup> Dehydroepiandrosterone sulfate was measured by direct radioimmunoassay as described by Buster and Abraham.<sup>9</sup> Statistical significances were de-

termined by Student's *t* test. Correlation coefficients were calculated between peripheral insulin levels and ovarian vein androgen levels.

### Results

The fasting insulin concentrations were increased above the normal range in all patients with hyperthecosis. Their mean fasting insulin concentrations ( $84 \pm 32 \mu\text{U/ml}$ ) were significantly higher than those found in normal women ( $8 \pm 1 \mu\text{U/ml}$ ;  $p < 0.05$ ) and in women with polycystic ovarian disease ( $14 \pm 2 \mu\text{U/ml}$ ;  $p < 0.05$ ) (Fig. 1). Fasting insulin levels in patients with polycystic ovarian disease were significantly higher than those in control subjects ( $p < 0.05$ ). Fig. 2 compares the insulin response to hyperglycemia in the three groups of women. The insulin response represents the sum of insulin concentrations at 1 hour, 2 hours, and 3 hours after oral glucose administration. The insulin response in women with ovarian hyperthecosis ( $1200 \pm 266 \mu\text{U/ml}$ ) was significantly higher than the response observed in patients with polycystic ovarian disease ( $333 \pm 56 \mu\text{U/ml}$ ;  $p < 0.01$ ) and in normal women ( $110 \pm 15 \mu\text{U/ml}$ ;  $p < 0.01$ ). The insulin re-

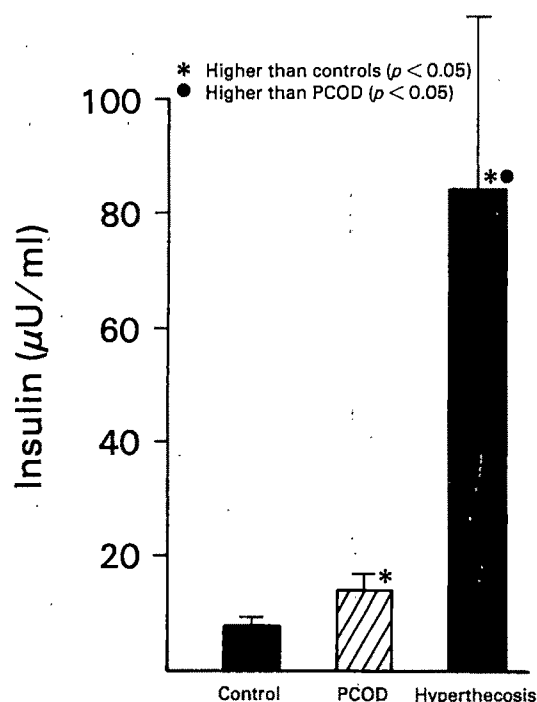


Fig. 1. Fasting insulin concentrations in control subjects and in patients with polycystic ovarian disease (PCOD) and those with hyperthecosis.

sponse in women with polycystic ovarian disease was higher than that in normal controls ( $p < 0.01$ ). However, the glucose response (the sum of glucose concentrations after an oral glucose tolerance test) was not significantly different among the three groups (Table II).

Concentrations of testosterone, dihydrotestosterone, and androstenedione in the patients with hyperthecosis were higher in the ovarian vein serum than in the peripheral vein, which indicates that the ovary is the source of these steroids (Table III). Dehydroepiandrosterone sulfate levels were normal, and no peripheral ovarian gradient was observed for this steroid. Significant positive correlations were found between peripheral insulin concentrations and ovarian testosterone ( $r = 0.879$ ,  $p < 0.02$ ), dihydrotestosterone ( $r = 0.866$ ,  $p < 0.03$ ), and androstenedione ( $r = 0.992$ ,  $p < 0.01$ ) (Fig. 3). There was no correlation between insulin levels and dehydroepiandrosterone sulfate. Histologic examination of the ovaries revealed nests of clear luteinized cells with foamy cytoplasm scattered within the stroma in all the six virilized patients (Fig. 4). Examination of these areas by frozen sections, and by oil red O stain for fat, demonstrated presence of lipid in the cytoplasm of these cells.

After the operation the levels of testosterone, dihydrotestosterone, and androstenedione returned to normal in all the patients. When an oral glucose tolerance test with insulin levels were repeated 3 months after

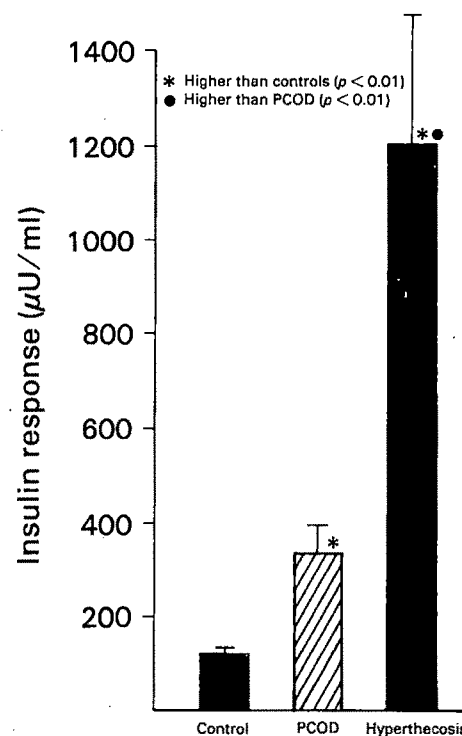


Fig. 2. Insulin responses to oral glucose tolerance tests in control subjects and in patients with polycystic ovarian disease and with hyperthecosis.

oophorectomy, insulin resistance persisted, with both fasting insulin concentrations and the insulin response to an oral glucose tolerance test significantly higher than in normal controls. The mean postoperative fasting insulin concentration of  $53 \pm 17$  µU/ml was lower than the mean preoperative level ( $84 \pm 32$  µU/ml), but the difference was not statistically significant. The insulin response to the oral glucose tolerance test was the same as the preoperative levels (Table IV). The mean postoperative glucose response of  $589 \pm 19$  mg/dl was also not significantly different from the preoperative response ( $578 \pm 50$  mg/dl). The body weights of the patients at the time of the oral glucose tolerance test after oophorectomy did not differ significantly from their weights before surgery.

#### Comment

Hyperandrogenism, in association with insulin resistance and acanthosis nigricans, has been referred to as HAIR-AN syndrome.<sup>10</sup> None of our patients, however, with hyperthecosis or polycystic ovarian disease had clinical features of acanthosis nigricans. Increased fasting insulin levels and exaggerated insulin response to oral glucose tolerance tests were observed in all the patients with hyperthecosis, and the levels were significantly higher in the hyperthecosis patients than in those with polycystic ovarian disease. This exaggerated

**Table II.** Serum insulin levels (basal and in response to oral glucose tolerance test)

Patient	Basal insulin level ( $\mu$ U/ml)	Sum of insulin levels ( $\mu$ U/ml)	Sum of glucose levels (mg/dl)
With hyperthecosis	$84 \pm 32^*$	$1200 \pm 266^\dagger$	$578 \pm 50$
With polycystic ovarian disease	$14 \pm 2^*$	$333 \pm 56^\dagger$	$488 \pm 45$
Control	$8 \pm 1$	$110 \pm 15$	$433 \pm 55$

\* $p < 0.05$ . $^\dagger p < 0.01$ .**Table III.** Peripheral and ovarian vein levels of androgens in patients with ovarian hyperthecosis (ng/dl)

Patient	Testosterone		Dihydrotestosterone		Androstenedione	
	Peripheral	Ovarian	Peripheral	Ovarian	Peripheral	Ovarian
1	219	4194	21	146	360	12414
2	207	816	28	74	193	4444
3	155	877	26	84	179	3310
4	150	610	19	63	160	2653
5	180	2213	32	120	290	4580
6	293	3273	40	101	102	5721
Mean $\pm$ SE	$201 \pm 21$	$1997 \pm 607$	$28 \pm 3$	$98 \pm 12$	$214 \pm 38$	$5520 \pm 1445$
Normal range	25-50		6-21		34-165	

**Table IV.** Fasting insulin levels and insulin response to oral glucose tolerance test in hyperthecosis patients before and after oophorectomy ( $\mu$ U/ml)

Patient	Basal insulin level		Sum of insulin levels	
	Preoperative	Postoperative	Preoperative	Postoperative
1	240	136	2370	1952
2	42	24	1360	2388
3	40	34	1240	1026
4	29	32	599	646
5	65	42	1005	872
6	88	54	625	761
Mean $\pm$ SE	$84 \pm 32$	$53 \pm 17$	$1200 \pm 266$	$1274 \pm 293$

insulin response in the presence of normal glucose levels indicates existence of insulin resistance in these patients. Many patients with hyperthecosis are obese, and obesity is associated with insulin resistance. The insulin resistance seen in these patients, however, is not entirely due to obesity, because the hyperthecosis patients were comparable to the control subjects in their body weights but had higher insulin levels. Similar observations have been made previously in women with polycystic ovarian disease. Chang et al.<sup>11</sup> observed insulin resistance in nonobese patients with polycystic ovarian disease. Shoupe et al.<sup>12</sup> observed higher insulin levels and higher peak insulin response during oral glucose tolerance tests in women with polycystic ovarian disease compared to weight-matched control subjects.

Previous studies indicate that significant positive correlations exist between insulin levels and the peripheral androgen levels in women with polycystic ovarian disease.<sup>13</sup> The significant correlation that is observed in

this study between peripheral insulin levels and ovarian vein testosterone, androstenedione, and dihydrotestosterone indicates that insulin stimulates ovarian secretion of these androgens. In normal women, dihydrotestosterone comes mainly from peripheral conversion and the ovary does not secrete any significant amount of this steroid.<sup>14</sup> The presence of a peripheral ovarian gradient for dihydrotestosterone in women with hyperthecosis indicates that hyperthecotic ovaries secrete significant amounts of dihydrotestosterone. During in vitro incubation studies of the ovarian stroma from a patient with HAIR-AN syndrome and stromal hyperthecosis, Barbieri et al.<sup>15</sup> observed that luteinizing hormone alone stimulated androstenedione and testosterone accumulation but not dihydrotestosterone accumulation and that insulin alone stimulated dihydrotestosterone accumulation as well. Since hyperthecotic ovaries secrete dihydrotestosterone, it is likely that insulin stimulates ovarian androgen production in these



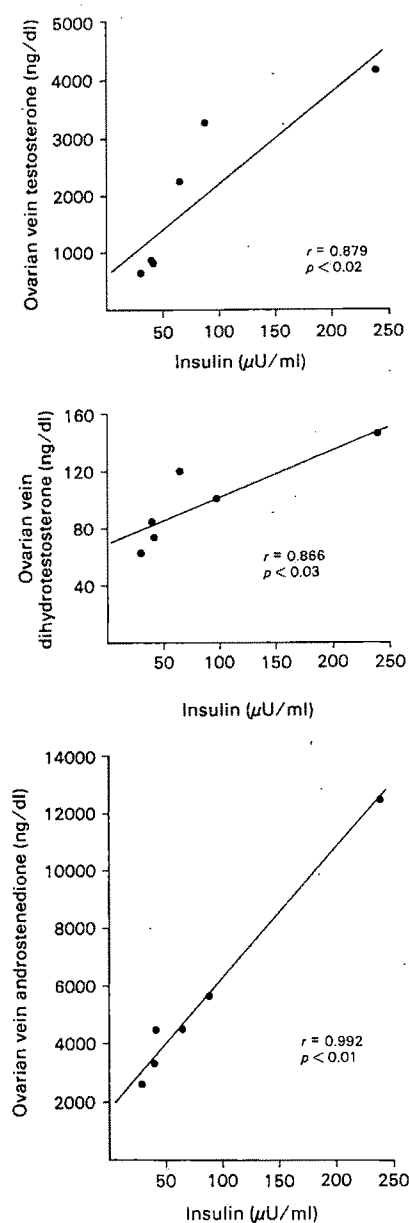


Fig. 3. Correlation between fasting insulin levels and ovarian vein testosterone, dihydrotestosterone, and androstenedione.

women in addition to luteinizing hormone. Specific high-affinity insulin receptors have been shown to be present in the ovarian stroma of patients with polycystic ovarian disease.<sup>16</sup> Women with insulin resistance have tissue resistance to the metabolic effects of insulin, but the effect of insulin on the ovarian stroma stimulating androgen synthesis seems to remain intact. The ovarian insulin receptors might be structurally or functionally different from the metabolic receptors present in other parts of the body. Further studies are needed to explore this possibility.

McNatty et al.<sup>17</sup> observed that the ovarian stromal tissue of a patient with insulin-resistant diabetes pro-

duced 49- to 250-fold more testosterone than did the stroma from normal ovaries. The effect of insulin on ovarian stromal cells might be to induce luteinization and convert them into steroidogenically more active luteinized stromal cells. Channing et al.<sup>18</sup> observed that luteinizing hormone- and follicle-stimulating hormone-induced luteinization of granulosa cells is enhanced by the inclusion of insulin in the medium, and increased lipid droplet accumulation was observed in insulin-treated cultures. Insulin might have a similar effect on the ovarian stromal cells as well. Insulin may stimulate lipid biosynthesis in the stromal cells, as one of its generalized metabolic effects, which might lead to lipid accumulation and luteinization of the cells. Infants of diabetic mothers, who are known to have hyperinsulinemia, have been reported to have increased numbers of luteinized cells in the ovarian stroma.<sup>19</sup> In our present study the patients who had the highest peripheral insulin levels were found, on histologic examination of the ovaries, to have the most extensive luteinization of the ovarian stroma and to have greatly elevated androgen levels in ovarian veins. The extent of luteinization of the ovarian stroma correlated somewhat with the peripheral insulin levels. The primary defect in patients with ovarian hyperthecosis might be insulin resistance, with a consequent increase in insulin levels, which in turn stimulates ovarian thecal and stromal androgen production. This could explain the common association of hyperandrogenism with insulin resistance.

The other possible explanation for this association of hyperandrogenism and insulin resistance is that insulin resistance may be a consequence of, or be secondary to, hyperandrogenism.<sup>11</sup> In the present study, insulin resistance persisted postoperatively when the androgen levels had returned to normal, which indicates that insulin resistance in these patients is not entirely due to hyperandrogenism. Previous data on the effect of hyperandrogenism on insulin resistance are limited, and women with HAIR-AN syndrome have been the subjects in all of these previous reports. The pathophysiology of insulin resistance in women with HAIR-AN syndrome might be different from that found in women with polycystic ovarian disease or hyperthecosis without acanthosis nigricans. In one patient, reported by Annos and Taymor,<sup>20</sup> hyperinsulinemia and acanthosis nigricans persisted after oophorectomy even after the androgen levels had returned to normal.

In summary, the results of this present study indicates that (1) a significant degree of insulin resistance exists in women with hyperthecosis that is not related to obesity; (2) the degree of insulin resistance is higher in patients with hyperthecosis than with polycystic ovarian disease; (3) insulin stimulates ovarian androgen

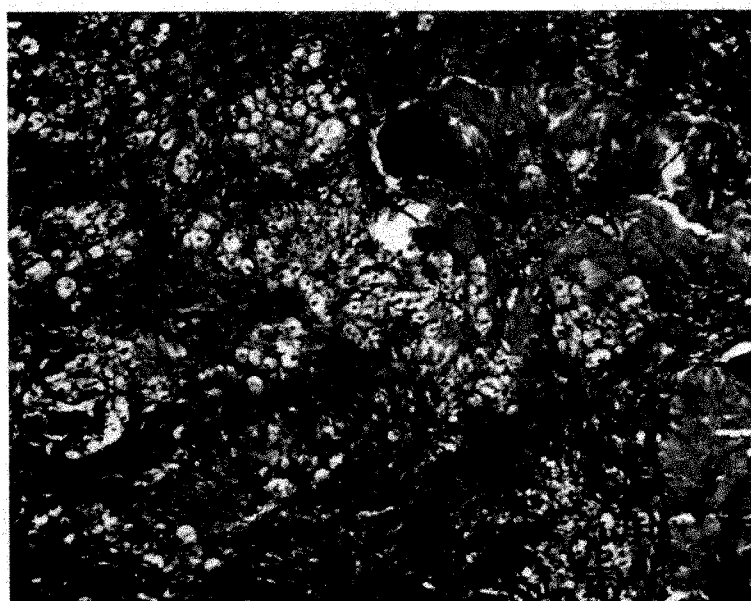


Fig. 4. Extensive stromal luteinization in patient 1.

production, possibly by inducing stromal luteinization, and thus may play a role in the pathogenesis of hyperthecosis; and (4) insulin resistance present in these women is not entirely due to hyperandrogenism because it persisted even when the androgen levels returned to normal after oophorectomy.

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# Demonstration of myc and ras oncogene expression by hybridization in situ in hydatidiform mole and in the BeWo choriocarcinoma cell line

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With the use of hybridization in situ, c-myc and c-ras oncogene expression has been identified in the cytotrophoblast of hydatidiform mole and in the malignant trophoblast cell line BeWo. Expression was also found in early villous placenta. No expression of these two oncogenes was found in the cytotrophoblast of an 11-week conceptus, nor was it found in term placenta. The significance of these findings is discussed. (AM J OBSTET GYNECOL 1986;154:390-3.)

**Key words:** Oncogenes, hybridization, hydatidiform mole, choriocarcinoma, BeWo cell line

Most acutely transforming ribonucleic acid tumor viruses carry genetic information called viral oncogenes that can induce neoplastic phenotypes in susceptible host cells.<sup>1,2</sup> These genetic sequences are homologous to endogenous host cell sequences called cellular oncogenes. They have been shown to be present in species as disparate as yeast, *Drosophila*, and man. They are normally activated during cell proliferation and division and very likely play fundamental roles in the rapid cellular proliferation of early embryonic development.<sup>2-4</sup> In adult cells, however, they appear to be tightly repressed. This repression can be disrupted by mutational events,<sup>5,6</sup> gene amplification,<sup>7,8</sup> or the acquisition of new regulatory sequences.<sup>9-11</sup> Such events lead either to overproduction of the oncogene messenger ribonucleic acid (RNA) and protein or to the production of abnormally "active" mutant gene products, resulting in uncontrolled cellular proliferation and other abnormal metabolic and physiologic derangements characteristic of a "neoplastic" phenotype.

Neoplastic cells often aberrantly express more than one oncogene. The evidence is more than circumstantial that the activity of multiple oncogenes is necessary to produce cells capable of invasive malignancy.<sup>12,13</sup> In such cases it is the activity of the first oncogene that confers the ability to proliferate indefinitely on primary cultures, while the action of a second or third oncogene may be necessary to produce invasively malignant cells.<sup>14</sup> In many respects, this stepwise acquisition of

"malignant" phenotypes is reminiscent of the stepwise graduations seen in many clinical situations, where a normal tissue gives rise to a hyperproliferative hyperplastic lesion, which may progress to an invasive malignant lesion. In this communication we present our observations on cell-specific oncogene expression in normal human trophoblast, hydatidiform mole, and choriocarcinoma BeWo cells, which represent a spectrum of benign to invasive malignant phenotypes. We have used hybridization in situ to localize oncogene overexpression to the cytotrophoblast elements.

The expressed oncogene may be identified by the use of radioactive probes homologous to the nucleic acid sequence of the oncogene. This may be done by gel electrophoresis and Southern blotting or by hybridization in situ.<sup>15,16</sup> In the technique of hybridization in situ, tissue specimens are treated with fixatives and denaturants to bind nucleic acid to the specimen and render it single stranded. Fixed tissue sections are then incubated with radioactive labeled probe deoxyribonucleic acid (DNA), so that hybridization of the probe with homologous nucleic acid fixed in the specimen can occur. The sections are washed to remove any unhybridized probe and coated with nuclear-track photographic emulsion. When the emulsion is developed, silver grains can be seen microscopically over those cells that contain nucleic acid sequences complementary to the probe. To assure that the binding is due to either DNA or RNA, adjacent sections can be digested with DNase or RNase and carried through the hybridization in situ as controls.<sup>16-19</sup>

## Patients and methods

Tissue from hydatidiform mole was obtained at the time of molar evacuation directly from the uterus so as

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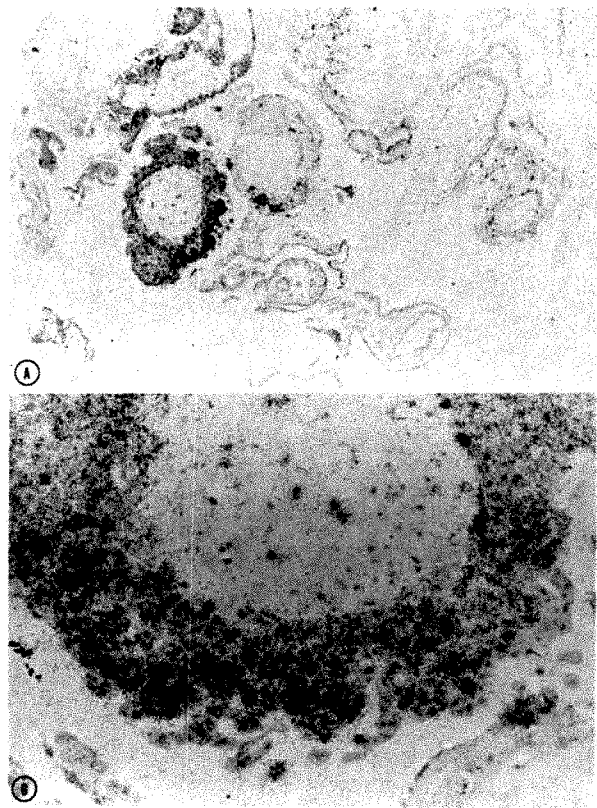
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to avoid the trauma that may be caused by the suction process. It was immediately fixed in a fresh solution of 4% paraformaldehyde with 0.5% glutaraldehyde in 0.1 mol/L sodium phosphate buffer at pH 7.5. After fixation at 4° C specimens were washed in 0.2 mol/L sodium phosphate buffer (pH 7.5) and 30% sucrose and processed on a Technicon automatic tissue processor. After these were embedded in paraffin, serial tissue sections of 6  $\mu$ m were cut and mounted on glass slides. Every tenth section was stained with hematoxylin and eosin for histologic examination. The remainder of the sections were used for studies of hybridization in situ.<sup>19</sup>

The sections were deparaffinized and hydrated through descending ethanol concentrations. Thin sections were digested with 1  $\mu$ g/ml of proteinase K in 0.1 mol/L Tris and 50 mmol/L ethylenediaminetetraacetic acid (EDTA) (pH 8.0) for 30 minutes at 37° C. After the sections were washed in distilled water, the slides were treated with acetic anhydride by the method of Hayashi et al.,<sup>20</sup> dehydrated, and air dried. For controls, slides were incubated with 100  $\mu$ g/ml of RNase A in 10 mmol/L Tris and 1 mmol/L EDTA (pH 8.0) for 60 minutes at 37° C immediately after the proteinase K digestion, before dehydration in ethanol.

For hybridization in situ, *Escherichia coli* strain plasmids containing the human c-myc gene and the v-H-ras were used. Plasmids were prepared by standard methods of cell lysis and CsCl buoyant density centrifugation. The cloned oncogene deoxyribonucleic acid fragments were prepared by agarose gel electrophoresis of an appropriate restriction digest of the chimeric plasmid and isolation of the oncogene fragment from the gel. The DNA fragment was radioactively labeled by nick translation with the use of sulfur 35-labeled adenosine triphosphate. <sup>35</sup>S was used in the place of the more usual <sup>32</sup>P-labeled nucleotide because of the tenfold lower energy and significantly smaller emulsion track length of the <sup>35</sup>S beta. This allows for better discrimination of the site of labeling than is possible with <sup>32</sup>P.<sup>19</sup> Each labeled DNA probe was dissolved in distilled water, denatured at 100° C for 2 minutes, and quick-cooled on ice. The solution was then made to include 25% formamide, 0.3 mol/L sodium chloride, 20 mmol/L Tris (pH 5.0), 5 mmol/L EDTA, 0.02% BSS, 0.02% Ficoll, 0.02% polyvinylpyrrolidone, and 50  $\mu$ g/ml of sheared denatured *E. coli* chromosomal DNA. Approximately 50  $\mu$ l of this hybridization mixture was applied to the dried slides, covered with siliconized glass coverslips, and incubated in a water-saturated atmosphere at 25° C overnight. The coverslips were removed and each slide was washed individually in 50 ml of 4X saline-sodium citrate solution for 10 minutes; 1X is 0.15 mol/L sodium chloride, 0.15 mol/L sodium citrate. They were pooled and washed for 15 minutes in 400 ml of 4X saline-sodium chloride solution and for



**Fig. 1.** A, Hydatidiform mole, grade 2. Hybridization in situ with myc oncogene probe showing strong expression of the oncogene. The <sup>35</sup>S grains are seen to be confined mainly to the cytotrophoblast. The expression with ras oncogene was less intense. B, Same tissue at higher power.

10 minutes in 400 ml of 2X saline-sodium chloride solution. Each slide was washed four times with 2.0 ml of 4X saline-sodium chloride solution. The slides were then pooled and washed, with stirring, for 30 minutes at 37° C in 4 L of 0.1X saline-sodium chloride solution and for 15 minutes, with stirring, at room temperature in 4 L of fresh 0.1X saline-sodium chloride solution.<sup>19</sup>

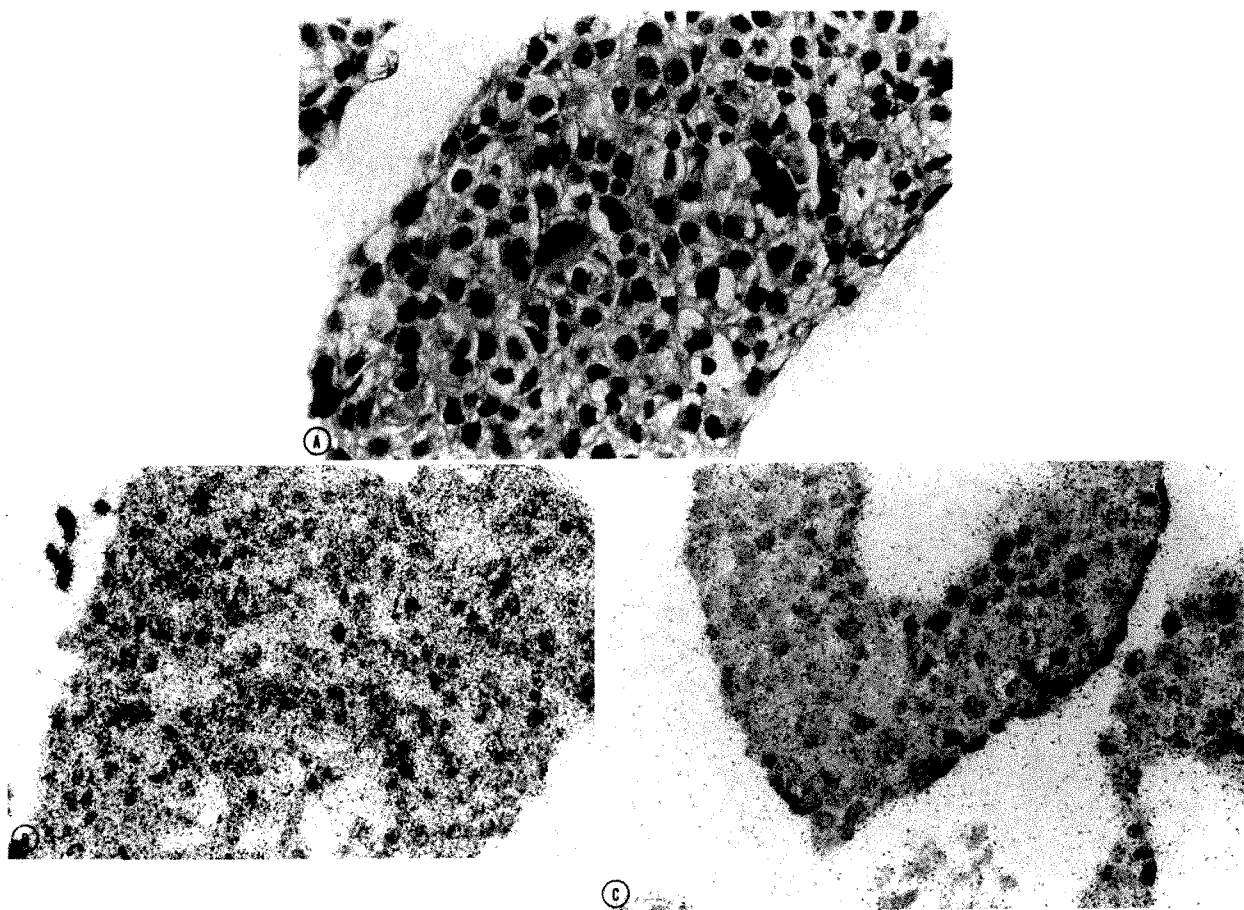
Autoradiography was performed after dehydration with ethanol. Slides were air-dried and exposed for 3 to 4 days, and stained with hematoxylin and eosin.

For normal controls in these experiments we used early villous trophoblast obtained from first-trimester terminations of pregnancy. The villous trophoblast was dissected free of core mesenchyme. The trophoblast specimens were obtained 4 and 8 weeks after conception. As a positive control we used cultures of the choriocarcinoma cell line BeWo, supplied by Dr. Roland Patillo. For negative controls we used term placentas obtained at the time of cesarean section.

## Results

Six hydatidiform moles have been examined so far and all demonstrated both c-myc and c-ras expression,





**Fig. 2.** A, Preparation of BeWo cultured choriocarcinoma cells (Hematoxylin and eosin.) B, BeWo choriocarcinoma cell line after hybridization in situ to demonstrate strong expression of myc oncogene. C, Same specimen as B with less intense expression of ras oncogene.

which appeared to be confined to the cytotrophoblast (Fig. 1). The expression of c-myc appeared to be significantly more masked than that of c-ras. There was marked expression of both oncogenes in the malignant trophoblast cell line BeWo (Fig. 2) and no expression in term placenta. Expression of c-myc and c-ras oncogene was found in early villous trophoblast at 4 weeks after conception and expression was not apparent at 8 weeks after conception. No expression was found in decidual tissue (Fig. 3) and none in term placenta.

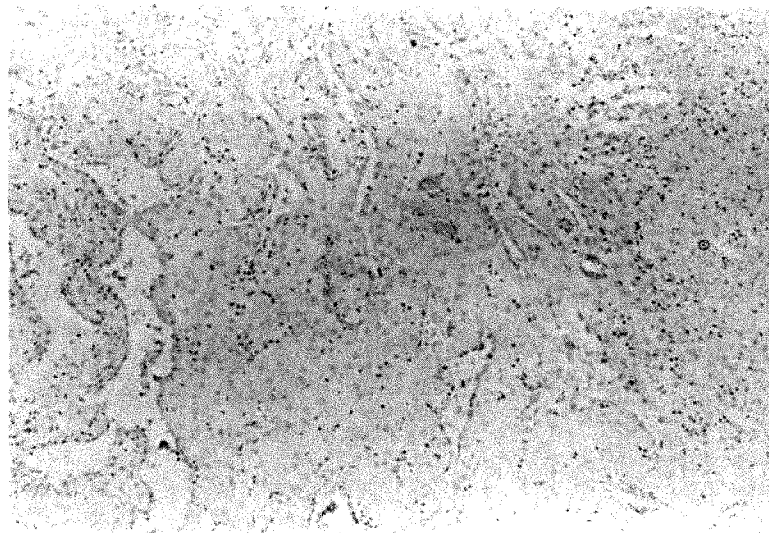
#### Comment

Sarkar et al.<sup>19</sup> have demonstrated hybridization of <sup>35</sup>S-labeled myc oncogene DNA to specific cells in preimplantation rat embryos.<sup>20</sup> Pfeiffer-Ohlsson et al.,<sup>21</sup> using Northern blot and hybridization in situ, have demonstrated the expression of human c-myc probes in early human villous cytotrophoblast with a peak expression at 5 weeks after conception and a decline by the end of the first trimester of pregnancy. They suggested a strong correlation between myc transcript abundance and trophoblastic proliferation. More recently, Goustin

et al.,<sup>22</sup> using a technique of hybridization in situ, demonstrated the expression of sis protooncogene, the structural gene for the B chain of platelet-derived growth factor, in first-trimester human cytotrophoblast.

Using the technique of hybridization in situ, we have demonstrated the expression of two cellular oncogenes, c-myc and c-ras, in the cytotrophoblast of hydatidiform mole and in the cells of the choriocarcinoma cell line BeWo. The expression of c-fos oncogene has previously been demonstrated by Northern blot analysis in this choriocarcinoma cell line.<sup>23</sup> C-myc oncogene expression has been previously demonstrated in early normal trophoblast with lack of expression by the end of the first trimester. The present findings are thus in agreement with those of Pfeiffer-Ohlsson et al.<sup>21</sup> Like these investigators, we failed to demonstrate oncogene expression in the term placenta.

The trophoblast of early placenta has many attributes of malignant tissue. It proliferates rapidly and "invades" the endometrium. Expression of certain cellular oncogenes may provide for rapid cell growth in the early trophoblast but it is currently still unknown how



**Fig. 3.** Decidual tissue in molar pregnancy from same patient shown in Fig. 1 to show absence of oncogene expression in nontrophoblastic tissue.

this physiologic oncogene expression differs from that seen during the malignant transformation of trophoblast to invasive mole and choriocarcinoma. It is the specific aim of our further studies to determine whether there may be a particular pattern of oncogene expression characteristic of invasive neoplasia. If such a pattern can be identified, we may have found a means to identify those hydatidiform moles that may be associated with malignant sequelae.

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# Effect of maternal hyperketonemia in hyperglycemic pregnant ewes and their fetuses

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The fetus of the pregnant diabetic woman is exposed to hyperglycemia frequently accompanied by ketoacidosis. Previous studies have demonstrated that  $\beta$ -hydroxybutyrate, a major ketone body, crosses the ovine placenta in significant amounts, leading to significant reductions in fetal  $\text{PaO}_2$  and increased fetal heart rate. In the present study the pregnant ewe was used to evaluate the maternal and fetal cardiovascular and metabolic responses to hyperketonemia in the presence of hyperglycemia and to determine if the combined diabetic insults were more detrimental to the fetus than hyperketonemia alone. A glucose priming dose of 25 gm was administered in the maternal femoral vein followed by a continuous glucose infusion of 200 mg/min to achieve steady maternal plasma glucose levels of 180 mg/dl. Once glucose levels were stable,  $\beta$ -hydroxybutyrate was infused for 2 hours at a rate of 0.39 mmol/100 ml of uterine blood flow into both left and right uterine arteries. Infusion of glucose alone did not significantly alter fetal cardiovascular and blood gas parameters but did increase the fetal glucose level from  $17 \pm 4$  to  $58 \pm 8$  mg/dl. The simultaneous infusion of  $\beta$ -hydroxybutyrate and glucose produced significant decreases in fetal  $\text{PaO}_2$  and oxygen content as were reported for hyperketonemia alone and significant time-related increases in fetal lactate levels and fetal heart rate. These data suggest that hyperketonemia in the pregnant ewe leads to quantitatively similar changes in oxygenation in both normoglycemic and hyperglycemic fetuses. These observations may in part help explain the increased perinatal mortality in the pregnant woman with uncontrolled diabetes. (AM J OBSTET GYNECOL 1986;154:394-401.)

**Key words:** Hyperglycemia, ketoacidemia, sheep, fetus

The fetus of a pregnant woman with poorly controlled diabetes is intermittently exposed to hyperglycemia and hyperketonemia. Maternal ketoacidosis has been shown to be associated with a higher incidence of perinatal loss<sup>1,2</sup> and impaired neurophysiologic development of the infant,<sup>3,4</sup> although the latter association is controversial.<sup>5</sup> Studies from our laboratory have shown that when the ketoacid  $\beta$ -hydroxybutyrate is administered to pregnant sheep, the ketone crosses the ovine placenta in small but significant amounts.<sup>6</sup> The experimental production of maternal hyperketonemia leads to a significant increase in fetal heart rate and a significant reduction in fetal  $\text{PaO}_2$  levels. These effects

are thought to be secondary to placental transfer of maternal ketones to the fetus since similar observations were noted when  $\beta$ -hydroxybutyrate was administered directly to the fetus.<sup>7</sup>

The present studies in pregnant sheep were undertaken to assess fetal cardiovascular and metabolic responses to maternal hyperketonemia in the presence of hyperglycemia and to determine if the combined diabetic insults are more detrimental to the fetus than hyperketonemia alone.

## Methods

**Animal preparation.** Operation was performed on 14 pregnant ewes (110 to 120 days' gestation) of mixed breed. Animals were sedated with diazepam, 10 mg intravenously, followed by thiopental sodium, 250 mg intravenously, before they received a hyperbaric spinal anesthetic (tetracaine hydrochloride, 12 mg). Polyvinyl catheters (0.05 inch by 0.09 inch) were implanted in the maternal femoral artery and vein and advanced to the distal aorta and inferior vena cava, respectively. After a sterile lower abdominal incision of 15 to 20 cm, the uterus was exposed and electromagnetic flow probes of the appropriate size were placed around each main uterine artery in the broad ligament. A lateral branch of both the right and left middle uterine arteries was catheterized with a polyvinyl catheter (0.04 inch by

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Group I	Saline	
Group II	Saline	Glucose
Group III	Saline	Glucose and Beta Hydroxybutyrate

Fig. 1. Experimental protocol.

0.07 inch) and advanced to the level of the bifurcation. A distal branch of both the right and left uterine veins was also catheterized with a polyvinyl catheter (0.04 inch by 0.07 inch) and advanced approximately 4 inches. The fetal neck was exposed via a small uterine incision, and the fetal carotid artery and jugular vein were cannulated with polyvinyl catheters (0.04 inch by 0.07 inch), which were advanced to the ascending aorta and superior vena cava, respectively. An open-ended catheter (0.125 inch by 0.250 inch) to obtain amniotic fluid samples was placed in the amniotic fluid. Ampicillin (1 gm) was administered into the amniotic fluid before uterine closure. All catheters and flow probe cables were exteriorized through the midline incision, passed subcutaneously to the ewe's left flank, and placed in a cloth pouch secured to the ewe's side. Fetal and maternal catheters were filled with heparin (100 and 1000 U/ml, respectively) and flushed daily to maintain patency. Ampicillin (1 gm) was administered intramuscularly to the pregnant ewe on the day of operation and for 3 days after operation. After operation, the animals were placed in portable cages and received water and commercial feed as desired. Ewes were allowed to recover from operation for 5 to 7 days before experimentation.

**Maternal and fetal measurements.** Samples for maternal and fetal arterial blood gas measurements ( $\text{PaO}_2$ ,  $\text{PCO}_2$ , pH) were collected anaerobically in heparinized syringes and immediately determined on a BMS3 Mk2 Micro System Blood Gas Analyzer (Radiometer, Copenhagen, Denmark) at 39° C. Oxygen content was also determined with the Lex-O<sub>2</sub>-ConTL Oxygen Content Analyzer (Cavitron, Waltham, Massachusetts). Plasma for  $\beta$ -hydroxybutyrate, lactate, and glucose determinations was separated by centrifugation and stored at -20° C until analysis.

$\beta$ -Hydroxybutyrate levels in fetal and maternal plasma and amniotic fluid were determined enzymatically according to the method of Williamson et al.<sup>8</sup> Amniotic fluid and plasma lactate was measured enzymatically according to the method of Hohorst.<sup>9</sup> The plasma glucose concentration was measured on a Beckman automatic glucose analyzer (Glucose Analyzer 2, Beckman Instrument Co., Fullerton, California).

Maternal and fetal systemic arterial blood pressures were monitored by Micron MP-15 blood pressure transducers (Micron Instruments, Los Angeles, California) attached to maternal and fetal arterial catheters. Heart

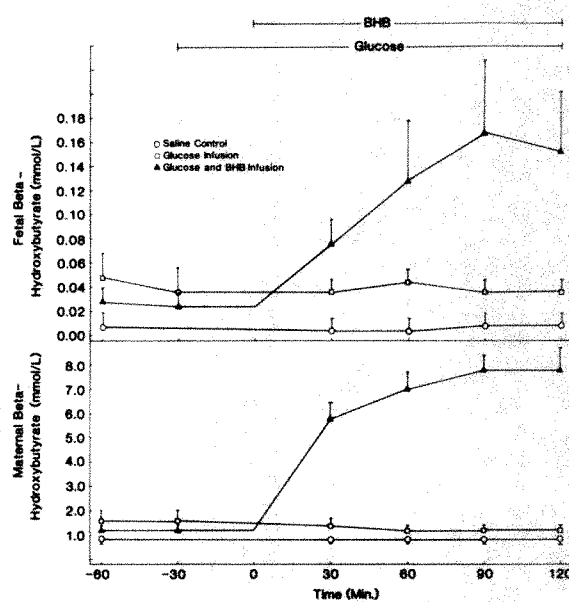


Fig. 2. Uterine venous (lower graph) and fetal (upper graph)  $\beta$ -hydroxybutyrate (BHB) levels in animals receiving  $\beta$ -hydroxybutyrate with glucose, glucose alone, or saline solution.

rates were recorded by a Beckman Cardiograph (Beckman Instruments, Fullerton, California) triggered by an arterial pressure pulse. Uterine arterial blood flow was measured by a square-wave electromagnetic flowmeter (Dienco RF-1000, Los Angeles, California). Electromagnetic flow probes were calibrated with saline solution before implantation and were linear over the blood flow ranges measured. Zero blood flow was recorded electronically on the flowmeters and previously verified during vascular occlusion. Blood pressure, heart rates, and blood flows were recorded continuously on a pen-writing recorder (Beckman Dynograph R-612 physiological recorder, Beckman Instruments, Fullerton, California).

Uterine oxygen uptake was calculated according to the Fick principle where uterine oxygen uptake is the product of uterine blood flow and the arteriovenous (maternal aorta and uterine vein) blood oxygen concentration difference.

**Experimental protocol.** The protocol consisted of a 30-minute period during which two blood samples were withdrawn at 15-minute intervals for baseline maternal and fetal arterial blood gas measurements, oxygen content, and glucose determinations and for maternal uter-



**Table I.** Effects of maternal infusion of saline solution, glucose, or  $\beta$ -hydroxybutyrate and glucose on maternal measurements

Maternal parameters	Baseline	Glucose or saline infusion period				
		0 min	BHB or saline infusion period			
			30 min	60 min	90 min	120 min
BHB, uterine vein (mmol/L)						
Group I (saline solution)	0.81 ± 0.15	—	0.81 ± 0.12	0.79 ± 0.13	0.79 ± 0.15	0.78 ± 0.17
Group II (glucose)	1.65 ± 0.40	1.56 ± 0.44	1.45 ± 0.31	1.30 ± 0.19	1.22 ± 0.17	1.21 ± 0.14
Group III (BHB and glucose)	1.29 ± 0.21	1.12 ± 0.22	5.73 ± 0.66*	7.03 ± 0.71*	7.79 ± 0.62*	7.78 ± 0.90*
Glucose, femoral artery (mg/dl)						
Group I (saline solution)	64 ± 6	—	68 ± 7	68 ± 7	68 ± 8	66 ± 9
Group II (glucose)	58 ± 5	206 ± 23*	183 ± 23*	172 ± 23*	166 ± 28*	171 ± 24*
Group III (BHB and glucose)	54 ± 3	208 ± 12*	185 ± 8*	178 ± 7*	178 ± 9*	171 ± 10*
Lactate, uterine vein (mmol/L)						
Group I (saline solution)	1.31 ± 0.48	—	1.23 ± 0.50	1.16 ± 0.38	1.04 ± 0.31	0.78 ± 0.22
Group II (glucose)	1.13 ± 0.14	1.27 ± 0.16	1.35 ± 0.15	1.16 ± 0.23	1.35 ± 0.09	1.40 ± 0.13
Group III (BHB and glucose)	1.68 ± 0.33	1.68 ± 0.46	2.03 ± 0.55	2.42 ± 0.61*	2.61 ± 0.51*	2.78 ± 0.50*
Heart rate (bpm)						
Group I (saline solution)	114 ± 3	—	113 ± 4	112 ± 5	113 ± 4	112 ± 3
Group II (glucose)	122 ± 7	122 ± 3	121 ± 5	125 ± 5	123 ± 4	124 ± 5
Group III (BHB and glucose)	107 ± 4	111 ± 5	118 ± 6	133 ± 6*	137 ± 5*	135 ± 6*
Mean arterial pressure (mm Hg)						
Group I (saline solution)	75 ± 4	—	75 ± 4	74 ± 4	74 ± 3	76 ± 5
Group II (glucose)	73 ± 4	71 ± 4	73 ± 5	73 ± 7	70 ± 6	65 ± 7
Group III (BHB and glucose)	75 ± 3	75 ± 3	76 ± 3	75 ± 4	75 ± 4	74 ± 4
Uterine blood flow (ml/min)						
Group I (saline solution)	904 ± 115	—	875 ± 97	866 ± 103	838 ± 118	843 ± 111
Group II (glucose)	696 ± 92	763 ± 48	772 ± 58	790 ± 57	831 ± 45	834 ± 56
Group III (BHB and glucose)	840 ± 104	888 ± 113	892 ± 111	856 ± 116	867 ± 98	823 ± 92
Oxygen content, femoral artery (vol/100 ml)						
Group I (saline solution)	9.9 ± 0.1	—	9.6 ± 0.2	9.6 ± 0.2	9.4 ± 0.3	9.4 ± 0.3
Group II (glucose)	9.6 ± 0.5	9.2 ± 0.8	9.2 ± 0.7	9.1 ± 0.4	9.4 ± 0.8	9.6 ± 0.9
Group III (BHB and glucose)	11.6 ± 0.7	11.0 ± 0.6	10.5 ± 0.4	10.6 ± 0.7	10.4 ± 0.6	11.0 ± 0.6
PaO <sub>2</sub> , femoral artery (mm Hg)						
Group I (saline solution)	103.9 ± 2.7	—	102.9 ± 2.1	102.1 ± 0.7	102.9 ± 1.1	101.1 ± 2.1
Group II (glucose)	101.1 ± 2.5	102.4 ± 1.4	106.8 ± 3.0	104.9 ± 3.2	108.9 ± 4.0	104.3 ± 3.8
Group III (BHB and glucose)	97.8 ± 2.1	102.0 ± 2.7	98.3 ± 3.2	93.8 ± 2.0	92.5 ± 5.0	95.9 ± 3.8
pH, femoral artery						
Group I (saline solution)	7.453 ± 0.018	—	7.474 ± 0.013	7.454 ± 0.023	7.461 ± 0.015	7.461 ± 0.013
Group II (glucose)	7.478 ± 0.025	7.513 ± 0.008	7.493 ± 0.008	7.472 ± 0.018	7.487 ± 0.018	7.490 ± 0.017
Group III (BHB and glucose)	7.483 ± 0.006	7.493 ± 0.012	7.506 ± 0.013	7.506 ± 0.019	7.524 ± 0.017	7.524 ± 0.012
PCO <sub>2</sub> , femoral artery (mm Hg)						
Group I (saline solution)	32.3 ± 1.1	—	30.3 ± 1.5	31.4 ± 2.4	31.0 ± 1.1	31.2 ± 0.8
Group II (glucose)	32.7 ± 2.6	29.5 ± 3.7	32.5 ± 2.6	29.9 ± 2.8	31.4 ± 3.7	31.9 ± 2.1
Group III (BHB and glucose)	32.4 ± 1.2	29.6 ± 1.8	30.7 ± 1.4	32.4 ± 0.9	33.9 ± 1.9	32.2 ± 1.5
Oxygen, uterine uptake (ml/min)						
Group I (saline solution)	36.6 ± 7.8	—	32.2 ± 7.6	33.4 ± 4.7	30.9 ± 9.6	31.9 ± 7.2
Group II (glucose)	18.5 ± 4.3	14.2 ± 0.7	17.9 ± 3.9	17.5 ± 4.5	18.7 ± 2.6	20.5 ± 1.3
Group III (BHB and glucose)	22.4 ± 2.2	26.9 ± 2.6	24.3 ± 5.2	24.5 ± 2.4	23.3 ± 2.6	26.5 ± 2.7

BHB =  $\beta$ -Hydroxybutyrate.\*Significantly different from baseline values ( $p \leq 0.05$ ).

ine vein and fetal arterial levels of  $\beta$ -hydroxybutyrate and lactate. Samples of amniotic fluid were also obtained during this time for baseline determinations of  $\beta$ -hydroxybutyrate, lactate, and glucose. Animals were

randomly assigned into one of three groups (Fig. 1) in which four animals were incorporated into group I, four animals into group II, and six animals into group III. Following the baseline period, an intravenous in-

**Table II.** Effects of maternal infusion of saline solution, glucose, or  $\beta$ -hydroxybutyrate and glucose on fetal measurements

Fetal parameters	Baseline	Glucose or saline infusion period				
		0 min	BHB or saline infusion period			
			30 min	60 min	90 min	120 min
BHB, carotid artery (mmol/L)						
Group I (saline solution)	0.01 ± 0.01	—	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
Group II (glucose)	0.05 ± 0.02	0.04 ± 0.02	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
Group III (BHB and glucose)	0.03 ± 0.01	0.03 ± 0.01	0.08 ± 0.02	0.13 ± 0.05*	0.17 ± 0.06*	0.15 ± 0.05*
Glucose, carotid artery (mg/dl)						
Group I (saline solution)	14 ± 1	—	16 ± 1	16 ± 1	16 ± 1	18 ± 1
Group II (glucose)	17 ± 4	71 ± 7*	63 ± 9*	60 ± 9*	59 ± 7*	58 ± 8*
Group III (BHB and glucose)	12 ± 2	53 ± 5*	58 ± 5*	59 ± 5*	56 ± 7*	57 ± 5*
Lactate, carotid artery (mmol/L)						
Group I (saline solution)	2.06 ± 0.41	—	2.06 ± 0.47	2.05 ± 0.46	2.16 ± 0.46	1.98 ± 0.40
Group II (glucose)	2.79 ± 0.57	2.97 ± 0.87	3.26 ± 0.77	3.05 ± 0.62	3.24 ± 0.81	3.94 ± 0.63
Group III (BHB and glucose)	2.70 ± 0.32	3.24 ± 0.53	3.78 ± 0.18	4.14 ± 0.20	6.96 ± 1.36*	6.80 ± 1.03*
Heart rate (bpm)						
Group I (saline solution)	147 ± 8	—	142 ± 8	150 ± 11	166 ± 4	159 ± 3
Group II (glucose)	169 ± 5	185 ± 13	179 ± 12	180 ± 13	180 ± 7	167 ± 7
Group III (BHB and glucose)	164 ± 7	169 ± 11	195 ± 15	187 ± 15	207 ± 14*	222 ± 12*
Mean arterial pressure (mm Hg)						
Group I (saline solution)	49 ± 3	—	49 ± 3	50 ± 4	49 ± 3	50 ± 3
Group II (glucose)	52 ± 2	52 ± 1	53 ± 2	52 ± 2	53 ± 1	52 ± 3
Group III (BHB and glucose)	55 ± 3	54 ± 2	56 ± 3	57 ± 3	59 ± 3	59 ± 4
PaO <sub>2</sub> , carotid artery (mm Hg)						
Group I (saline solution)	22.6 ± 1.2	—	23.7 ± 1.4	21.5 ± 2.8	22.2 ± 1.2	22.4 ± 1.6
Group II (glucose)	22.4 ± 1.6	23.3 ± 2.2	21.7 ± 1.9	20.9 ± 1.8	21.3 ± 2.0	20.9 ± 1.5
Group III (BHB and glucose)	22.7 ± 1.7	20.9 ± 2.0	19.2 ± 2.5	18.1 ± 1.9*	18.1 ± 1.9*	17.5 ± 1.9*
Oxygen content, carotid artery (vol/100 ml)						
Group I (saline solution)	8.0 ± 0.6	—	7.9 ± 1.0	6.9 ± 0.8	7.4 ± 1.1	7.3 ± 0.6
Group II (glucose)	6.8 ± 0.5	6.3 ± 0.7	6.2 ± 0.5	6.2 ± 0.4	6.1 ± 0.6	5.9 ± 0.9
Group III (BHB and glucose)	6.7 ± 0.7	5.6 ± 0.9	4.6 ± 0.8	3.9 ± 0.6*	3.7 ± 0.7*	3.5 ± 0.6*
pH, carotid artery						
Group I (saline solution)	7.354 ± 0.019	—	7.370 ± 0.016	7.369 ± 0.021	7.363 ± 0.019	7.374 ± 0.023
Group II (glucose)	7.399 ± 0.010	7.396 ± 0.015	7.400 ± 0.007	7.384 ± 0.007	7.385 ± 0.012	7.373 ± 0.011
Group III (BHB and glucose)	7.384 ± 0.006	7.381 ± 0.007	7.361 ± 0.017	7.330 ± 0.029	7.327 ± 0.038	7.317 ± 0.029
PCO <sub>2</sub> , carotid artery (mm Hg)						
Group I (saline solution)	42.2 ± 2.0	—	44.4 ± 2.9	42.3 ± 4.2	44.1 ± 3.4	44.7 ± 1.7
Group II (glucose)	42.2 ± 4.0	42.2 ± 4.7	41.1 ± 3.7	45.1 ± 2.7	44.6 ± 4.2	45.5 ± 3.0
Group III (BHB and glucose)	43.5 ± 2.3	43.5 ± 2.2	45.6 ± 2.4	46.2 ± 1.8	47.9 ± 3.3	46.1 ± 1.9

BHB =  $\beta$ -Hydroxybutyrate.\*Significantly different from baseline values ( $p \leq 0.05$ ).

jection of 25 gm of glucose (groups II and III) or an equal volume of saline solution (group I) was administered into the maternal femoral vein followed by a continuous infusion of glucose (200 mg/min) or an equal volume of saline solution. Glucose levels were maintained between 160 and 210 mg/dl in groups II and III. Thirty minutes after this infusion,  $\beta$ -hydroxybutyrate (group III) or an equal volume of saline solution (groups I and II) was infused directly into the uterine arteries for a period of 2 hours.  $\beta$ -Hydroxybutyrate (Sigma Chemical Co., St. Louis, Missouri) was

infused in its buffered form at a rate of 0.39 mmol/100 ml of uterine blood flow. Fetal (2 ml) and maternal (3 ml) arterial blood samples and maternal uterine vein (3 ml) and amniotic fluid (3 ml) samples were obtained at 15-minute intervals throughout the study period for blood gas and metabolic determinations. Maternal and fetal heart rate and blood pressure, as well as uterine blood flow, were monitored throughout the experimental period.

**Statistical analysis.** The data analysis was designed to detect differences between experimental groups and

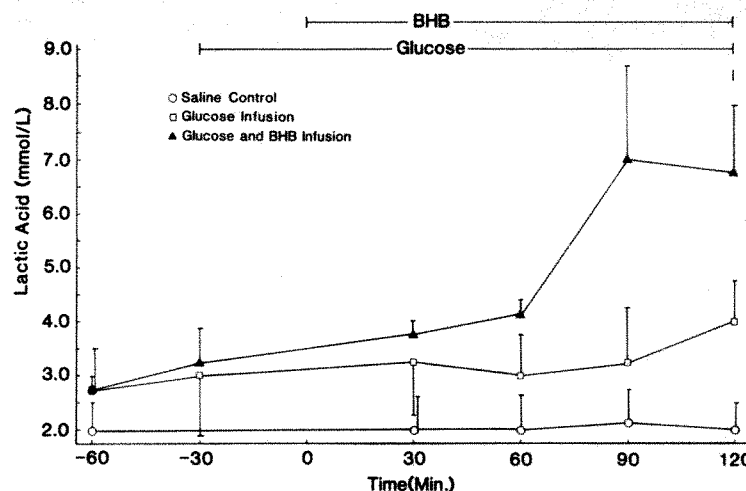


Fig. 3. Fetal lactic acid versus time in animals receiving  $\beta$ -hydroxybutyrate (BHB) and glucose, glucose alone, or saline solution.

differences across time and to ascertain whether the experimental groups produced the same pattern across time (test for interaction or parallelism). Baseline values were the average of two separate blood sample determinations obtained at 15-minute intervals. Each reading thereafter was then expressed as a percentage of the baseline value. The logs of the percentages were used to obtain normally distributed data for statistical analysis. Data were analyzed by means of repeated measures analysis of variance to detect group effects, time effects, and time-group interaction. A test for sphericity was conducted at the time of analysis, and if found to be significant, the Greenhouse-Geisser correction was made in testing for time effects and time-group interactions. If the time-group interaction was significant, F tests were conducted to determine the significance of the time effect within each group. If the time-group interaction was not significant but the group effect was significant, F tests were conducted to determine differences between groups.<sup>10</sup> Results are expressed as the mean  $\pm$  SE. The acceptable level of significance was established at  $p \leq 0.05$ .

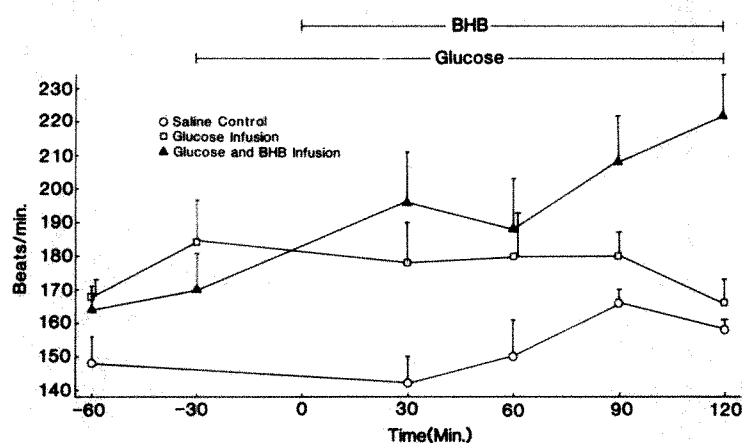
## Results

**Maternal measurements.** The simultaneous administration of  $\beta$ -hydroxybutyrate and glucose (group III) produced significant increases in maternal uterine vein  $\beta$ -hydroxybutyrate and lactate concentrations, maternal femoral artery glucose concentrations, and maternal heart rate. Maternal uterine vein  $\beta$ -hydroxybutyrate levels increased from  $1.29 \pm 0.21$  to  $7.78 \pm 0.90$  mmol/L (Fig. 2). Maternal femoral artery glucose concentrations rose from  $54 \pm 3$  to  $171 \pm 10$  mg/dl, which is comparable to the increase in glucose levels produced in animals receiving glucose without  $\beta$ -hydroxybutyrate (group II; Table I). Maternal uterine vein lactate levels

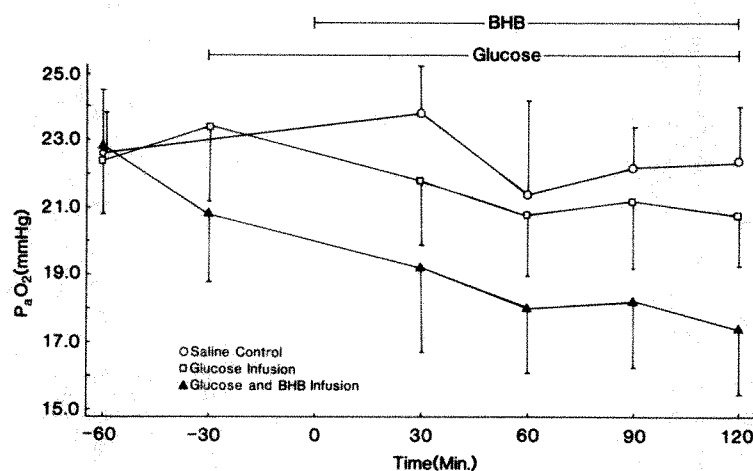
increased from  $1.68 \pm 0.33$  to  $2.78 \pm 0.50$  in group III whereas this change in lactate was not observed in the other two groups (Table I). Maternal heart rate increased significantly from  $107 \pm 4$  to  $135 \pm 6$  bpm when  $\beta$ -hydroxybutyrate and glucose were coadministered whereas no change in maternal heart rate was noted in the glucose-treated (group III) or in the control saline solution-treated (group I) animals (Table I).

Maternal mean arterial pressure, uterine blood flow, blood gas values, oxygen content, and uterine oxygen uptake did not significantly change throughout the experimental period in any of the study groups (Table I).

**Fetal measurements.** The coadministration of  $\beta$ -hydroxybutyrate and glucose (group III) led to significant changes in fetal arterial  $\beta$ -hydroxybutyrate, glucose, and lactate concentrations as well as in fetal heart rate,  $PaO_2$ , and oxygen content. The fetal arterial  $\beta$ -hydroxybutyrate concentration increased from  $30 \pm 10$  to  $170 \pm 60$   $\mu$ mol/L (Fig. 2, Table II). The glucose changes seen in group III were comparable to those seen in group II, in which glucose concentrations significantly increased from  $12 \pm 2$  to  $57 \pm 5$  mg/dl (Table II). Fetal arterial lactate levels rose from  $2.70 \pm 0.32$  to  $6.80 \pm 1.03$  mmol/L (Fig. 3) in group III whereas there were no significant changes in lactate levels in the other two groups (Table II). The increases in fetal lactate in the hyperglycemic ewe receiving ketones occurred much earlier and were higher than those observed in the previous study in which only ketones were infused.<sup>7</sup> Fetal heart rate increased significantly from  $164 \pm 7$  to  $222 \pm 12$  bpm (Fig. 4, Table II), a change not noted in the glucose-treated (group II) or saline solution-treated (group I) animals (Table II). Fetal  $PaO_2$  and oxygen content were significantly decreased during the simultaneous infusion of  $\beta$ -



**Fig. 4.** Fetal heart rate changes versus time in animal receiving  $\beta$ -hydroxybutyrate (BHB) with glucose, glucose alone, or saline solution.



**Fig. 5.** Fetal arterial  $P_{aO_2}$  versus time for animals receiving  $\beta$ -hydroxybutyrate (BHB) and glucose, glucose alone, or saline solution.

hydroxybutyrate and glucose. Fetal  $P_{aO_2}$  decreased from  $22.7 \pm 1.7$  to  $17.5 \pm 1.9$  mm Hg (Fig. 5, Table II) and oxygen content from  $6.7 \pm 0.7$  to  $3.5 \pm 0.6$  vol/100 ml (Fig. 6, Table II). These changes were not evident in the other two groups.

Fetal mean arterial pressure, pH, and  $PCO_2$  (Table II) and amniotic fluid levels of  $\beta$ -hydroxybutyrate, glucose, and lactate (Table III) were not significantly altered in any of the study groups throughout the experimental period.

#### Comment

The production of maternal hyperketonemia and hyperglycemia by the infusion of  $\beta$ -hydroxybutyrate and glucose induced significant increases in maternal and fetal heart rate and in plasma ketone ( $\beta$ -hydroxybutyrate), glucose, and lactate concentrations. The increase in fetal  $\beta$ -hydroxybutyrate concentration was associated with a reduction in  $P_{aO_2}$  and oxygen content.

Previous studies from our laboratory have demonstrated that the infusion of  $\beta$ -hydroxybutyrate alone led to equivalent changes in fetal  $P_{aO_2}$ .<sup>7</sup> The elevation of maternal and fetal  $\beta$ -hydroxybutyrate levels in previous studies produced increases in maternal lactate levels and in maternal and fetal heart rate as seen in the present study. In the present study, the fetal lactate levels began to increase by 60 minutes after the beginning of the ketone infusion in contrast to the previous study where fetal lactate levels increased at 120 minutes after the beginning of the ketone infusion. Fetal lactate levels reached a peak of  $6.96 \pm 1.36$  mmol/L in the present study whereas they increased only to  $4.16 \pm 1.05$  mmol/L in previous studies with the infusion of ketones alone. This change in fetal lactate was associated with a nonsignificant decrease in fetal pH. It is unclear if longer ketone and glucose infusion would have resulted in continued accumulation of lactate and further reduction in fetal pH. The combined



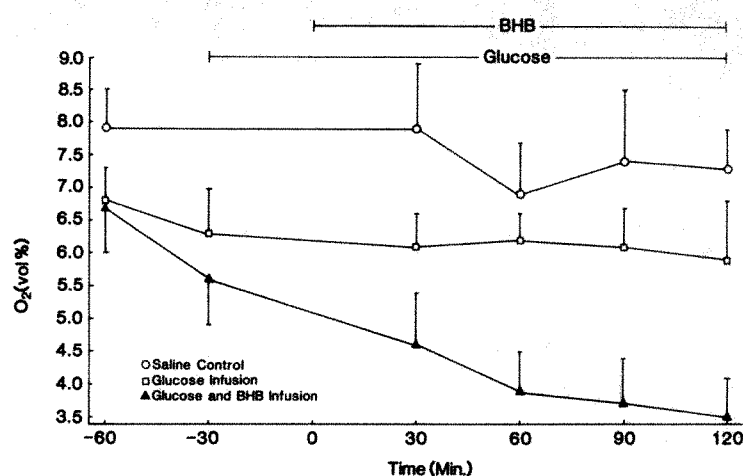


Fig. 6. Fetal arterial oxygen content versus time for animals treated with  $\beta$ -hydroxybutyrate (BHB) and glucose, glucose alone, or saline solution.

Table III. Effects of maternal infusion of saline solution, glucose, or  $\beta$ -hydroxybutyrate and glucose on amniotic fluid measurements

Amniotic fluid parameters	Baseline	Glucose or saline infusion period				
		0 min	BHB or saline infusion period			
			30 min	60 min	90 min	120 min
BHB (mmol/L)						
Group I (saline solution)	0	0	0	0	0	0
Group II (glucose)	0.04 ± 0.002	0.04 ± 0.003	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.003
Group III (BHB and glucose)	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01
Lactate (mmol/L)						
Group I (saline solution)	1.37 ± 0.55	—	1.74 ± 0.77	1.63 ± 0.58	1.48 ± 0.65	1.51 ± 0.58
Group II (glucose)	2.92 ± 0.94	2.31 ± 0.93	2.55 ± 0.51	2.63 ± 0.65	2.76 ± 0.70	2.77 ± 0.60
Group III (BHB and glucose)	2.72 ± 0.74	2.03 ± 0.52	1.96 ± 0.53	2.02 ± 0.53	2.40 ± 0.58	2.52 ± 0.53
Glucose (mg/dl)						
Group I (saline solution)	13 ± 2	—	11 ± 1	12 ± 1	12 ± 2	11 ± 1
Group II (glucose)	13 ± 4	13 ± 6	11 ± 3	12 ± 3	13 ± 2	12 ± 2
Group III (BHB and glucose)	12 ± 2	12 ± 2	13 ± 1	13 ± 2	13 ± 1	14 ± 2

BHB =  $\beta$ -Hydroxybutyrate.

diabetic insult of hyperketonemia and hyperglycemia did not prove to be more detrimental to the fetus during the 2-hour period as assessed by cardiovascular, blood gas, and metabolic measurements. It therefore appears that hyperketonemia in the pregnant ewe leads to changes in fetal cardiovascular and metabolic measurements with similar degrees of such changes in both normoglycemic and hyperglycemic fetuses.

The production of maternal hyperglycemia, by the infusion of glucose, significantly elevated maternal and fetal plasma glucose levels but did not produce any other significant effect on maternal or fetal cardiovascular, metabolic, or blood gas measurements. These results are in agreement with reports by Shelly et al.,<sup>11</sup> who found no evidence that hyperglycemia was harmful to a well-oxygenated fetus, producing only a small increase in the plasma lactate level if the plasma glucose level was raised above 40 mg/dl. However, if the fetus was slightly hypoxic and the glucose level was raised

above 40 mg/dl, there were rapid increases in fetal lactate levels with corresponding decreases in pH. Robillard et al.<sup>12</sup> found that fetal lactate levels increased and blood pH decreased only when fetal glucose levels were >150 mg/dl and fetal  $P_{O_2}$  decreased only when glucose levels exceeded 300 mg/dl. Infusion of glucose alone (group II) did not result in any significant changes in fetal lactate,  $P_{aO_2}$ , oxygen content, or pH values. These findings are in agreement with studies by Shelley et al.<sup>11</sup> and Robillard et al.,<sup>12</sup> since the fetuses in the present study were not hypoxic and the glucose levels were <150 mg/dl. More recent studies by Philipps et al.<sup>13</sup> have shown that direct infusion of glucose into the fetus for 2 to 17 days led to a lowering of fetal arterial oxygen content. Although the level of glucose in the present study was comparable to that produced by Philipps et al. (60 mg/dl), the duration of the infusion was only 2 hours, which is substantially shorter than in the studies of Philipps et al. It therefore appears

that short-term moderate elevations in glucose do not adversely affect fetal cardiovascular, blood gas, or metabolic parameters unless the fetus is already hypoxic prior to the rise in glucose.

The mechanisms responsible for the effects produced by  $\beta$ -hydroxybutyrate are still speculative. The  $\beta$ -hydroxybutyrate-induced increase in maternal and fetal heart rate may be due to a direct effect of  $\beta$ -hydroxybutyrate on the heart or suggestive of catecholamine release subsequent to  $\beta$ -hydroxybutyrate infusion or catecholamine release subsequent to  $\beta$ -hydroxybutyrate-induced lowering of fetal oxygen content.  $\beta$ -Hydroxybutyrate may lead to an increase in lactate levels resulting from an accumulation of pyruvate from  $\beta$ -hydroxybutyrate metabolism and the need for an alternate pathway because of a low capacity for oxidative metabolism. Jones and Robinson<sup>14</sup> have shown that fetal hypoxia results in elevated plasma catecholamine and lactate levels.<sup>15</sup> In turn, catecholamines are known to increase fetal heart rate<sup>14, 16</sup> and lactate levels.<sup>17</sup> Therefore, two mechanisms may be responsible for the observed fetal and maternal cardiovascular and lactate changes—either a direct ketone effect and/or the release of catecholamines because of  $\beta$ -hydroxybutyrate or  $\beta$ -hydroxybutyrate-induced fetal hypoxia.

The cause of the  $\beta$ -hydroxybutyrate-induced reduction in fetal oxygenation is currently unclear. Several possible mechanisms may be responsible for the  $\beta$ -hydroxybutyrate-induced alteration in fetal oxygenation. One mechanism may be that the fetus is consuming more oxygen and consequently reducing the level of oxygen measured. Another possibility is that the transfer of oxygen from maternal hemoglobin to fetal hemoglobin is diminished, resulting in lower fetal oxygen levels. Finally, the placenta may be consuming more oxygen or placental blood flow may be compromised, which leads to reduced oxygen delivery to the fetus.

The present study suggests that amniotic fluid levels of  $\beta$ -hydroxybutyrate, lactate, and glucose may not accurately reflect fetal exposure to these compounds. The amniotic fluid levels of  $\beta$ -hydroxybutyrate, glucose, and lactate did not increase significantly even though blood levels of these compounds were markedly elevated. Additional time may be required for these compounds to appear in the amniotic fluid; however, measurements of these compounds in amniotic fluid do not appear to reflect the blood levels.

The present results indicate that acute moderate elevations of plasma glucose levels in well-oxygenated fetuses do not adversely affect fetal cardiovascular,

blood gas, or metabolic parameters. In contrast, ketones are able to reduce fetal oxygenation and to elicit specific cardiovascular (increase in heart rate) and metabolic (increase in lactate) changes during coexisting hyperglycemia. These studies confirm changes that were previously observed during  $\beta$ -hydroxybutyrate infusion to normoglycemic pregnant ewes.<sup>17</sup> It therefore appears that hyperketonemia in the pregnant ewe leads to alterations in cardiovascular and metabolic status in both normoglycemic and hyperglycemic fetuses and may account for the increased perinatal mortality in the patient with uncontrolled diabetes.

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# Pharmacologic levels of nitrendipine do not affect actin-myosin interaction in the human uterus and placenta

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Because of the potential of dihydropyridine calcium channel blockers in the management of premature labor, we have studied the direct effects of nitrendipine on actomyosin in the pregnant and nonpregnant uterus and in the term human placenta. Actomyosin adenosinetriphosphatase in the three tissues and another model of actin-myosin interaction, superprecipitation of placental actomyosin, were inhibited by nitrendipine. The inhibition was not diminished by high concentrations of calcium. To identify the mechanism, placental myosin was phosphorylated in the absence and presence of  $0.8 \times 10^{-4}$  mol/L of nitrendipine. The myosin phosphorylated in the presence of nitrendipine had lower actin-activated adenosinetriphosphatase, which is consistent with the inhibition of myosin light chain phosphorylation. However, nitrendipine did not affect the adenosinetriphosphatase activity of myosin nor did further reduce the adenosinetriphosphatase of the already phosphorylated placental actomyosin. Thus nitrendipine inhibition is directed to the phosphorylation reaction but not to the adenosinetriphosphatase site of myosin. Myometrial relaxation in vivo or in vitro occurs at the pharmacologic nitrendipine levels of  $10^{-9}$  to  $10^{-8}$  mol/L, which is at least 10,000 times lower than that of the concentration of 50% inhibition of myosin light chain phosphorylation ( $0.0026 \pm 0.00015$  mol/L of nitrendipine, mean  $\pm$  SEM) demonstrated in the present work. Because of this difference, the direct intracellular actions of dihydropyridine calcium channel blockers are not expected to cause adverse effects in the uteroplacental system when these drugs are used in the prevention or treatment of premature labor. (AM J OBSTET GYNECOL 1986;154:402-7.)

**Key words:** Calcium channel blockers, nitrendipine, myosin light chain phosphorylation, actomyosin of uterus and placenta

Contractility, in both skeletal and smooth muscles, is regulated by the levels of intracellular free calcium. Calcium channel blockers, including nitrendipine [3-ethyl-5-methyl-1,4-dihydro-2,6-dimethyl-1-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate], inhibit voltage-regulated calcium channels and diminish muscle contraction in proportion with site-specific binding of the drugs.<sup>1,3</sup> The muscle-relaxant properties of calcium channel blockers are used in various cardiac indications,<sup>4,5</sup> but several preliminary studies suggest that these drugs will also have a role in prevention and treatment of premature labor or menses-related uterine hypercontractility in nonpregnant women.<sup>6-11</sup> We have previously demonstrated in parturient rats that calcium channel blockers inhibit labor in a statistically significant manner by extending uterine confinement

and by elongating the time elapsed between delivery of pups.<sup>12</sup> Nitrendipine appears to act directly on the myometrium, because the decline in serum progesterone levels, which is a result of complex events preceding labor in rats, was identical in both the control and in the nitrendipine-treated animals.<sup>13</sup>

In smooth muscles, such as the myometrium or placenta, the interaction of actin and myosin is regulated by phosphorylation of the 20,000 mW myosin light chains.<sup>14</sup> A complex of calcium and calmodulin is necessary to activate the myosin light chain kinase, which causes increased myosin light chain phosphorylation, more actin-myosin interaction, and thus increased smooth muscle contractility (see model in Fig. 4). While it is well documented that the primary action of calcium channel blockers is directed to the inhibition of calcium influx across muscle membranes,<sup>3,5,15</sup> several reports indicate that dihydropyridines or their metabolites enter the cell<sup>16</sup> and, in addition to the blockage of the calcium transport, may have direct effects on the contractile apparatus. For instance, dihydropyridines appear to inhibit the contractile activity of membrane-free smooth muscle preparations,<sup>17</sup> and action of the various verapamil derivatives seems to be related to their ability to cross the muscle cell membrane.<sup>18</sup> Dihydropyridines

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also bind to calmodulin and were reported to block calmodulin-dependent activation of phosphodiesterase,<sup>19</sup> while calmodulin antagonists also inhibit the binding of nitrendipine to the cell membrane.<sup>20</sup>

Because of the potential of calcium channel blockers in tocolytic management, we decided to investigate the effects of nitrendipine on the actomyosin preparations from the human pregnant and nonpregnant uterus and from the human placenta. In the latter we have previously demonstrated that the degree of myosin light chain phosphorylation and the level of the actomyosin interaction are directly related.<sup>21,22</sup> Intracellular effects of these drugs may be very important because, in addition to the myometrium, the muscles of the blood vessels and of the placental anchoring villi are also regulated by myosin light chain phosphorylation and thus could be affected by calcium channel blockers. We have found that nitrendipine does not affect the adenosinetriphosphatase (ATPase) properties of uterine or placental myosins, but it inhibits the phosphorylation of myosin light chains, thus the activity of actomyosin ATPase. However, this inhibition occurs only at concentrations of about  $10^4$ -fold higher than that of the  $10^{-9}$  to  $10^{-8}$  mol/L pharmacologic dose in which calcium channel blockers inhibit myometrial contractility. Thus intracellular action of dihydropyridines in pharmacologic levels are not likely to affect placental or fetal functions if used in tocolytic management.

#### Material and methods

All chemicals used were of analytical grade and were obtained from Mallinckrodt Chemical Works and from Fischer Scientific Company. Chromatography supplies were from Eastman Chemical Company and from Pharmacia. Protein standards, dithiothreitol, and phenylmethylsulfonyl fluoride were supplied by Sigma Chemical Company. Nitrendipine was received from the Miles Institute for Preclinical Pharmacology, New Haven, Connecticut.

The preparation of actomyosin and myosin from pregnant and nonpregnant uteri and from term placenta and the assays for ATPase activities was carried out as described previously,<sup>21,22</sup> except that the ammonium sulfate fractionation was omitted. Phosphorylation (and dephosphorylation) of myosin light chains occurs in this preparation because myosin light chain kinase and myosin light chain phosphatase copurify with the actomyosin.

Myosin light chain phosphorylation was carried out in 0.6 mol/L of potassium chloride, 20 mmol/L of imidazole (pH 7.0), 0.1 mmol/L calcium chloride, 1.5 mmol/L of magnesium chloride, and 1 mmol/L ATPase at room temperature with or without nitrendipine. The phosphorylation assays were started with the addition

of adenosine triphosphate (ATP) and were terminated after 5 minutes by adjusting the ethylenediaminetetraacetate (EDTA) concentrations to 20 mmol/L. The phosphorylated actomyosin was chromatographed on a Sepharose 4B column, which separates actomyosin and myosin from the other proteins, including the myosin light chain kinase and phosphatase.

Conditions for myosin ATPase determinations were as follows. Potassium-activated ATPase: 5 mmol/L of EDTA and 0.6 mol/L of potassium chloride; actin-activated ATPase: 0.6 mmol/L of calcium chloride, 1.5 mmol/L of magnesium chloride, and 0.06 mol/L of potassium chloride. All assays were 1.0 ml volume and contained about 20 to 50  $\mu$ g of protein, 1 mmol/L of ATP, and 20 mmol/L of imidazole buffer (pH 7.0); they were incubated at 37° C for 30 minutes. The released inorganic phosphate was determined as described earlier.<sup>21</sup>

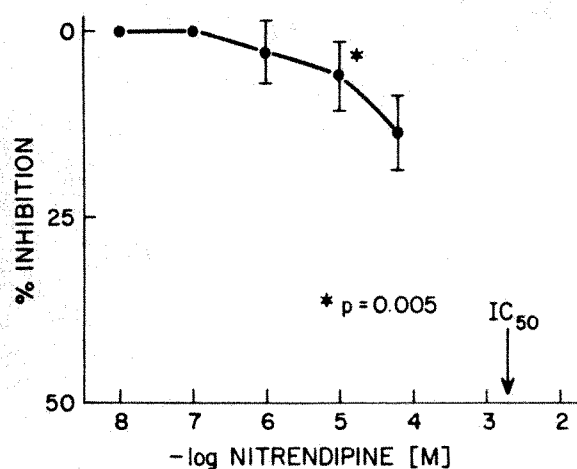
Conditions for superprecipitation were as follows: 0.5 mg/ml of actomyosin, 1.5 mmol/L magnesium chloride, 0.06 mol/L potassium chloride, 2 mmol/L ATP, and either 0.02 mmol/L of calcium chloride or 2 mmol/L of ethyleneglutaminetetraacetate (EGTA). The reaction was started with the addition of ATP and the increase in turbidity was observed at 660 nm.

#### Results

**Solubility of nitrendipine.** Dihydropyridines, including nitrendipine, have low solubility in aqueous solutions, but they dissolve well in ethanol and in polyethylene glycol. Since an organic solvent is necessary to keep nitrendipine in the assay solution, we tested the effects of various ethanol and polyethylene glycol concentrations on myosin. The mean of five experiments indicated that the losses of ATPase activity caused by ethanol (percent of ethanol concentrations are in parentheses) were as follows: 3% (0.5), 5% (1.0), 6% (1.5), 9% (2.0), 12% (3.0), 19% (4.0), and 33% (5.0), respectively. Polyethylene glycol was more damaging than ethanol at comparable concentrations. Thus we decided to use 2% ethanol because it caused only about 10% loss of ATPase activity, while the saturation point for nitrendipine was  $0.8 \times 10^{-4}$  mol/L as determined by the absorption at 235 nm.

**Nitrendipine inhibition of ATPase.** ATPase activity of actomyosin and myosin in the nonpregnant and pregnant uterus and in the human placenta were determined in a series of increasing nitrendipine concentrations. Myosin ATPase was unaffected; however, actomyosin ATPase was inhibited, and the inhibition became significant at  $10^{-5}$  mol/L of nitrendipine (Fig. 1). Because actomyosin ATPase in smooth muscles is based on both the integrity of the ATPase site of myosin and on the phosphorylation of the regulatory myosin light chains, we further investigated whether nitrendipine inhibits the ATPase activity of myosin or whether it

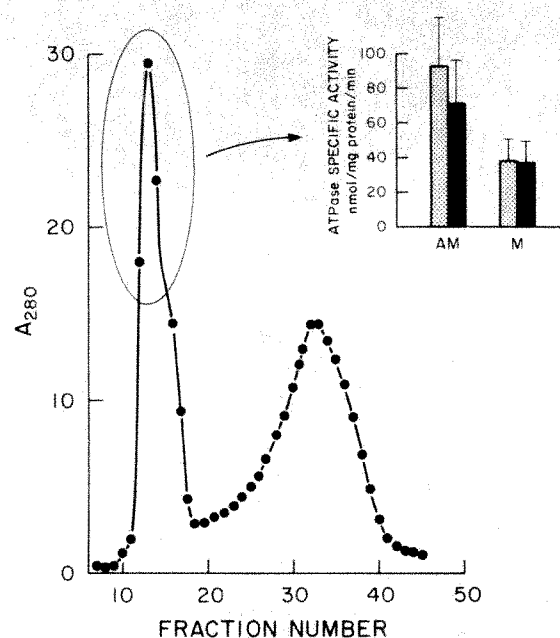




**Fig. 1.** The effects of nitrendipine on the ATPase activity of uterine and placental actomyosin. Actomyosins from the pregnant and nonpregnant human uterus and from term human placenta were prepared and the ATPase activities were determined in the presence of  $0$ ,  $10^{-8}$ ,  $10^{-7}$ ,  $10^{-6}$ ,  $10^{-5}$ , and  $0.8 \times 10^{-4}$  mol/L concentrations of nitrendipine as indicated. The data points represent the mean of eight, seven, six, seven, seven, and eight determinations, respectively, on individual uterine and placental tissue samples. Nitrendipine inhibition occurs at  $10^{-6}$  mol/L concentrations, and it becomes significant at  $10^{-5}$  mol/L ( $p = 0.005$  according to the paired  $t$  test). The 50% inhibition concentration ( $IC_{50}$ ), based on all experiments, extrapolates to  $0.0026 \pm 0.00015$  (SEM) mol/L of nitrendipine as determined by the Allfit program of DeLean, Munson, and Rodbard (National Institutes of Health, Bethesda, Maryland).

interferes with the myosin light chain phosphorylation reaction.

**Effect of nitrendipine on myosin light chain phosphorylation.** To examine the effects of nitrendipine, placental actomyosin was prepared and myosin light chain phosphorylation was carried out in the presence and absence of  $0.8 \times 10^{-4}$  mol/L of nitrendipine. The ATPase activities of column-purified actomyosin and myosin fractions were determined in the actomyosin and myosin ATPase conditions. The elution pattern of the Sepharose 4B columns, and the ATPase activities of the respective fractions (determined in duplicates of five independent phosphorylation experiments) are shown in Fig. 2. (All data are reported as mean  $\pm$  SD.) ATPase activity of actomyosin, phosphorylated in the absence of nitrendipine, was  $93.4 \pm 27.0$  nmol of inorganic phosphate per milligram of protein per minute, whereas the sample phosphorylated in the presence of nitrendipine had significantly lower activities of  $71.6 \pm 24.7$  nmol of inorganic phosphate per milligram of protein per minute ( $p = 0.002$ ). The myosin ATPase activities were not affected by nitrendipine; the values were  $38.5 \pm 12.6$  and  $37.3 \pm 12.8$  nmol of inorganic phosphate per milligram of protein per minute ( $p = 0.22$ ), respectively. The data suggest that the de-

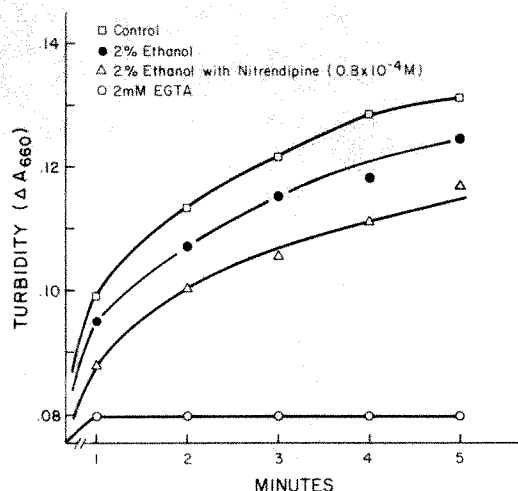


**Fig. 2.** The effects of nitrendipine on myosin light chain phosphorylation. Actomyosin preparations from the human placenta were phosphorylated in the presence and absence of  $0.8 \times 10^{-4}$  mol/L of nitrendipine, and were subsequently chromatographed on Sepharose 4B columns as described in Material and methods. Fractions 13, 14, and 15 were pooled, and ATPase activities for actomyosin and myosin were determined in the samples. The insert shows the results of five such experiments. Each determination was carried out in duplicate on a different placental preparation with use of the respective column fractions for the ATPase assays. Actomyosin ATPase activities were  $93.4 \pm 27.0$  and  $71.6 \pm 24.7$  nmol of inorganic phosphate per milligram of protein per minute ( $n = 5$ ) in the nitrendipine-treated and control samples, respectively (dotted and black bars). The inhibition was significant ( $p = 0.002$ , by  $t$  test or by two-way analysis of variance). The myosin ATPase activities were not affected ( $38.5 \pm 12.6$  versus  $37.3 \pm 12.8$  nmol of inorganic phosphate per milligram of protein per minute  $p = 0.22$ ).

cline in ATPase activity following nitrendipine treatment is due to the inhibition of the myosin light chain kinase system. Increased concentrations of calcium (up to 5 mmol/L) in the phosphorylation media did not alleviate the nitrendipine inhibition. To further demonstrate that nitrendipine does not have a direct effect on actin or myosin, we have added nitrendipine to the column-purified actomyosin and observed no additional inhibition of either actomyosin or myosin ATPase.

#### Superprecipitation in the presence of nitrendipine.

Another model of actin-myosin interaction is the superprecipitation phenomenon in the presence of ATP. Fig. 3 summarizes a typical superprecipitation experiment in four conditions: control, with 2% ethanol, with 2% ethanol and  $0.8 \times 10^{-4}$  mol/L of nitrendipine, and in the absence of calcium (2 mmol/L of EGTA). Ethanol



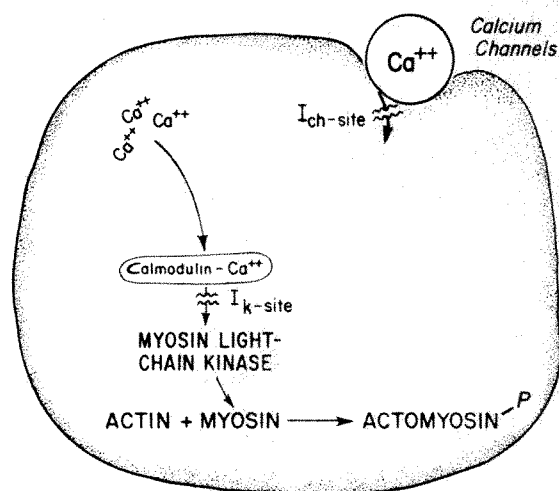
**Fig. 3.** Placental actomyosin superprecipitation. A typical superprecipitation experiment was carried out as described under Material and methods. Nitrendipine inhibits the superprecipitation as compared to the ethanol control. In the absence of calcium (5 mmol/L of EGTA) the increase in turbidity is almost completely abolished.

reduced the actomyosin turbidity by about 5% compared to the control, the nitrendipine caused a further decrease of about 9%, and finally in the absence of calcium (2 mmol/L of EGTA) the turbidity was only about 20% of that of the control value. The relative ATPase activities in the four conditions were 113%, 100%, 80%, and 6%.

### Comment

Calcium channel blockers, which inhibit calcium influx and muscle contractility, are increasingly used in various fields of medicine and research.<sup>3-5</sup> We have previously examined the effects of nitrendipine on parturient rats and found a significant delay in the delivery of the first pup, as well as in the time period elapsed in deliveries of the subsequent pups.<sup>12</sup> We have also monitored progesterone plasma levels and demonstrated that administration of nitrendipine has not affected the endocrine events underlying labor.<sup>13</sup> In vivo effects of other calcium channel blockers have been demonstrated previously in the relaxation of spontaneous or prostaglandin-mediated uterine contractility.<sup>10, 11</sup> In isolated myometrial strips there was a dose-related inhibition of spontaneous or oxytocin-initiated contractility, and the strips have become fully inhibited at about  $10^{-9}$  mol/L concentrations.<sup>23, 24</sup>

Several recent reports indicate that calcium channel blockers of the dihydropyridine type enter cells and that they may act intracellularly. For instance, nifedipine inhibits contractility in membrane-free muscle fibers,<sup>17</sup> and in different systems it was demonstrated that the calcium channel blockers bind to calmodulin



**Fig. 4.** The sites of nitrendipine action in smooth muscles. Actin-myosin interaction, and thus muscle contractility, is regulated by the phosphorylation of myosin light chains. The key enzyme of the process, the myosin light chain kinase is activated by the complex of calcium-calmodulin. Calcium enters the muscle cells through the calcium channels. The primary action site of the calcium antagonists, such as nitrendipine, are the calcium channels ( $I_{ch}$ -site), in which inhibition occurs at the pharmacologic levels of about  $10^{-9}$  to  $10^{-8}$  mol/L. Calcium channel blockers also interfere with the binding of the calmodulin-calcium complex to the myosin light chain kinase ( $I_k$ -site) at about  $10^{-3}$  to  $10^{-4}$  mol/L of nitrendipine concentrations, which are at least 1000-fold higher. Thus the latter type of inhibition is not likely to play a role in the uteroplacental system when calcium channel blockers are used in tocolytic management or for other indications. (Modified from Fig. 7 in Huszar G. Cellular regulation of myometrial contractility and essentials of tocolytic therapy. In: Huszar G, ed. The Physiology and Biochemistry of the Uterus in Pregnancy and Labor, Boca Raton, Florida: CRC Press, 1986; with permission.)

and inhibit the activation of various enzymes (for example, phosphodiesterase or phospholamban) by the calcium-calmodulin complex.<sup>19, 25</sup> Since calcium channel blockers may have a potential role in the therapy for premature labor, we investigated whether direct nitrendipine inhibition of the actomyosin system would adversely affect the smooth muscle of uterus and placenta.

Studying actomyosin of the human pregnant and nonpregnant uterus and of the placenta, we found that nitrendipine inhibits actomyosin ATPase. The inhibition became significant at  $10^{-5}$  mol/L. When all the inhibition data in Figs. 1 and 2 were considered, the curve extrapolates to a 50% inhibition concentration of  $0.0026 \pm 0.00015$  mol/L of nitrendipine. To further establish whether the inhibitory action is directed to myosin light chain phosphorylation or to the ATPase of myosin, we have phosphorylated human placental myosin in the presence and absence of nitrendipine. In this system we have previously demonstrated the

close correlation between myosin light chain phosphorylation and actin-myosin interaction, as it is measured by the increase in actin-activated myosin ATPase.<sup>22</sup> After chromatographic purification of the actomyosin fractions, we found that nitrendipine inhibited the activated ATPase at a significant level, which is consistent with the inhibition of myosin light chain phosphorylation. Another measure of actin-myosin interaction, superprecipitation, was also consistently inhibited by nitrendipine.

The target site of nitrendipine thus was localized in the myosin light chain kinase system, because phosphorylation in the presence of nitrendipine caused the inhibition of actomyosin ATPase, while the ATPase of myosin was not affected. Also, nitrendipine added to the already actin-activated, thus phosphorylated, myosin did not cause further inhibition.

As these in vitro experiments take place in the absence of the cell membrane and calcium channels, there are two possible steps of kinase activation that nitrendipine may diminish (Fig. 4). Either it blocks the formation of the calcium-calmodulin complex by covering the calcium sites of calmodulin or it may inhibit the activation of myosin light chain kinase by the calcium-calmodulin complex. Apparently, the latter mechanism is valid. Nitrendipine inhibited the binding of calcium-calmodulin complex to the myosin light chain kinase in the turkey gizzard system, and in agreement with our experience, higher calcium concentration did not override this inhibition.<sup>26</sup> Furthermore, the 50% inhibition concentration of nitrendipine inhibition was  $10^{-1}$  mol/L, which is similar to our value in the uterine and placental actomyosin system, as well as to the 50% inhibition concentration of nifedipine of another calmodulin-activated enzyme, phosphodiesterase.<sup>19</sup> Calcium channel blockers do not disturb the active site of myosin light chain kinase; rather, their effects are directed to the calmodulin-regulatory site of the kinase. When the latter site was removed by limited proteolytic digestion, myosin light chain kinase inhibition by nitrendipine was markedly diminished.<sup>27</sup>

The question arises whether the 15% to 20% inhibition of actomyosin ATPase observed at  $0.8 \times 10^{-4}$  mol/L of nitrendipine concentrations has any functional significance in vivo. The answer is almost certainly no, because calcium channel blockers would fully inhibit muscle contractility via the membrane action ( $I_{ch}$ -sites, Fig. 4) at concentrations of about 1000 to 10,000 times lower than the nitrendipine levels that inhibit myosin light chain phosphorylation ( $I_k$ -site). In fact, in recent experiments<sup>11, 23, 24</sup> significant inhibition of both electrical and mechanical activities of the myometrium was demonstrated with  $10^{-8}$  mol/L of nitrendipine or nifedipine. These data, along with the clinical experience of delayed labor in animals and humans,<sup>6, 9-13</sup> sug-

gest that calcium blockers of the dihydropyridine types will be a safe and useful drug in the management of preterm labor.

There is a need for newer approaches in tocolytic therapy because the cardiovascular and metabolic side effects of our primary agent, the  $\beta$ -adrenergic agonists, cause concern. The calcium channel blockers are viewed with expectation, although it is not clear whether they will be important in the stabilization of patients with premature labor or as a maintenance drug following  $\beta$ -adrenergic therapy.<sup>28</sup> The question with respect to maternal and fetal side effects of calcium channel blockers arises because of their relaxing action on vascular smooth muscles.<sup>29</sup> When the systematic vascular resistance diminishes, there is a secondary decrease in blood pressure and a reflex compensatory increase in heart rate and cardiac output in the mother. If calcium channel blockers are combined with  $\beta$ -adrenergic drugs, the negative inotropic effects of nitrendipine may increase the risk for congestive failure. This problem may be further compounded in patients who are candidates for operative procedures (for example, cesarean section) because narcotics and inhalation anesthetics may interfere with the compensatory tachycardia.<sup>30</sup> More data are also needed on the effects of calcium channel blockers on the fetal circulation, although nitrendipine, which is water insoluble, is not likely to cross the placenta.

In any case, our study has clearly defined the membrane and intracellular effects of calcium channel blockers in the uteroplacental system. The data demonstrate that the interference with the cellular apparatus of smooth muscles is not a clinical concern, because it occurs at concentrations of at least 1000 times higher than the pharmacologic inhibition of smooth muscle contractility.

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# Improving the yield of direct chorionic villus slide preparations

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Low cytogenetic yields in the processing of small chorionic villus sampling have in some instances, limited its applicability. We have modified "standard" techniques to increase the number of interpretable metaphases by (1) using a gravity method for cell suspension spreading and (2) using a special high-quality slide glass. Both modifications reduce cell damage and increase interpretable mitotic figures and may allow a cytogenetic diagnosis in some instances in which a diagnosis might not otherwise be possible with standard methods. (AM J OBSTET GYNECOL 1986;154:408-11.)

**Key words:** Chorionic villus sampling, cytogenetic diagnosis, chromosome banding

Chorionic villus sampling holds great promise for the detection of both cytogenetic and biochemical abnormalities in the first-trimester fetus. However, there are still some technical difficulties in the processing of the sample that can limit its clinical applicability. In some instances, the sample may be too small to allow cytogenetic diagnosis. Clearly, experience is the major determinant of laboratory success. However, in some instances, limitations of sample size require more than standard yields to permit a diagnosis. Therefore, it is important to maximize the yield of the number of interpretable mitotic figures that can be obtained from any tissue sample. Several modifications of the "original" technique as outlined by Simoni et al.<sup>1,2</sup> and Szabo et al.<sup>3</sup> have been used to increase chromosome banding quality. Examples include changes in hypotonic treatment and cold storage of the cells at 4° C in the fixative overnight.<sup>4</sup> We have approached the technical problem of improving cytogenetic results for chorionic villus sampling specimens from the perspective of maximizing interpretable preparations from a given amount of tissue, which is important when sample size is limited. We hypothesized that careful handling of specimens should reduce cell damage and therefore increase yields. Specifically, we have tested separately two alterations in slide preparation techniques. The methodologic alterations tested were: (1) using gravity to allow the cell suspension to spread over the slides versus the more commonly used "rake" method and (2) using an extremely high-quality glass, as compared with standard slide glass.

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## Material and methods

Chorionic villus samples were obtained, with appropriate informed consent, from 10 patients undergoing first-trimester pregnancy terminations (five for each study). Aliquots of 10 mg of villi were carefully separated exclusively by one investigator from the other tissues under a dissecting microscope and were transferred to Dulbecco's phosphate-buffered saline. Clean villi were then transferred to culture tubes containing RPMI 1640 (Gibco No. 320-1875) solution, supplemented with 20% bovine serum and 0.003% antibiotic and antimycotic mixture. After 24 hours, the culture medium was removed with a micropipette and the villi were treated with 1% sodium citrate for 5 minutes. After the sodium citrate solution was drawn into a micropipette, the cell mixture was treated with Carnoy's fixative (3:1 methanol:acetic acid). After 10 minutes the fixative was removed, and a 0.5 ml solution of 60% acetic acid in water was added.

All laboratory manipulations were performed by one investigator. All specimens were split for a matched pair study, and data were blindly collected by another investigator.

For the purposes of this study, an intact metaphase contained 46 chromosomes, a hypomodal metaphase had more than the haploid number but less than 46 chromosomes, and fragments were defined as cells containing less than 23 chromosomes. Numbers of fragments were calculated but excluded from statistical analysis because damage to any one metaphase could yield a variable number of fragments. Comparisons of the numbers of intact and hypomodal cells from each specimen were performed and blinded as to the method of preparation of each slide. The mean numbers of intact and hypomodal cells per slide per method were calculated, as well as the proportions of intact to



Fig. 1A. Low-power magnification of Freed glass showing multiple imperfections.

total metaphases. Paired *t* tests were used for statistical analysis, with  $p < 0.05$  considered significant.

**Method of slide preparation.** A slide with six drops of the cell suspension was placed on a slide warmer ( $40^{\circ}\text{C}$ ) at a 10-degree incline and the solution was allowed to run slowly (gravity method) across the slide. Next, with a 90-degree rotation, the suspension was allowed to flow back to give an even spread. In the "rake" method, six drops from the same suspension of cells were spread on the slide with a curved glass pipette.

**Slide quality.** To test the hypothesis that the number of intact metaphases can be increased by reducing the surface roughness of the slides, we compared preparations on a very high-quality glass slide obtained through Carl Zeiss Canada Ltd. with those on the standard "Freed" glass slide supplied by American Scientific Products (Figs. 1A and 1B). The Freed slides are routinely used to perform cytogenetic studies on amniotic fluid cultures in our laboratory.

Table I. Effect of method of slide preparation on metaphase quantity

Method	Intact metaphases* (mean $\pm$ SD)	Hypomodal metaphases† (mean $\pm$ SD)	Proportion of intact to total metaphases‡ (mean $\pm$ SD)
Gravity	46.4 $\pm$ 9.8	69.0 $\pm$ 13.5	0.40 $\pm$ 0.25
Rake	14.0 $\pm$ 5.0	44.4 $\pm$ 13.6	0.24 $\pm$ 0.12

All figures are expressed as means per slide.

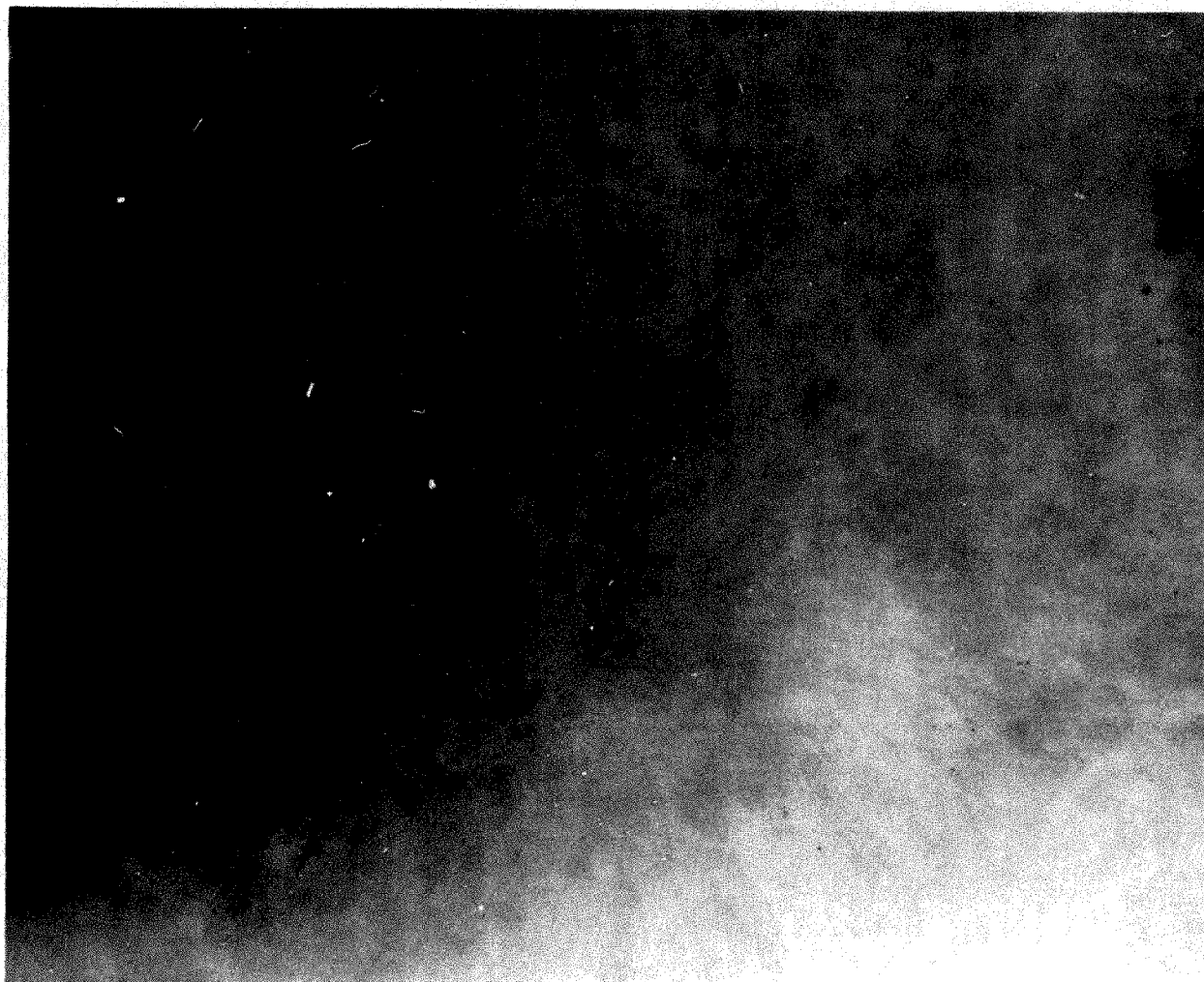
\**t* = 8.40;  $p < 0.05$ .

†*t* = 3.13;  $p < 0.05$ .

‡*t* = 6.37;  $p < 0.01$ .

## Results

**Gravity versus rake.** For this portion of the study, a total of 960 mitotic figures were examined on Schott glass. As shown in Table I, the gravity method consistently yielded higher numbers of total metaphases than did the rake method, with the numbers of intact and hypomodal cells both significantly increased with the



**Fig. 1B.** Low-power magnification of Schott glass showing fewer imperfections. Slide placement was random.

**Table II.** Effect of glass slide quality on metaphase quantity

Type of slide	Intact metaphases* (mean $\pm$ SD)	Hypomodal metaphases† (mean $\pm$ SD)	Proportion of intact to total metaphases‡ (mean $\pm$ SD)
Schott	56.6 $\pm$ 26	25.6 $\pm$ 21.6	0.74 $\pm$ 0.08
Freed	36.6 $\pm$ 12.7	59.3 $\pm$ 38.5	0.42 $\pm$ 0.09

All figures are expressed as means per slide.

\* $t = 4.20$ ;  $p < 0.05$ .

† $t = 3.99$ ;  $p < 0.05$ .

‡ $t = 9.94$ ;  $p < 0.01$ .

former method. Moreover, the proportion of intact to total mitotic figures was higher in the gravity method.

**Slide quality.** To compare the effect of the quality of the glass slides, 999 mitotic figures were examined on the two types of slides. As shown in Table II, the smoother Schott glass slide preparation yielded a

higher number of intact metaphases than the standard Freed glass slide. The number of hypomodal metaphases was reduced with Schott glass. The average proportion of intact to total cells was 0.74 with Schott and 0.42 with the Freed glass slides.

The cytogenetic quality of the intact metaphases was judged to be comparable in all groups, varying between 250 and 400 band levels.

### Comment

Chorionic villus sampling is an appealing method for the early prenatal diagnosis of cytogenetic disorders. Given that the safety of the procedure is confirmed, it may be expected to play an important role in the management of the pregnancies at increased risk for an aneuploid fetus. However, tissue quantities obtained by chorionic villus sampling are often limited and, in some cases, there may not be enough material to accomplish the cytogenetic analysis. Also, in some situations, a part

of the specimen may have to be submitted for either biochemical or deoxyribonucleic acid analysis of villi, thereby further reducing the amount of tissue available for cytogenetic study. In these situations, maximizing the yield of the intact metaphases may play an important role in being able to arrive at a cytogenetic diagnosis.

It is reasonable to suspect that more gentle cell handling will reduce cell damage, that is, the number of mitotic figures lost because of cell breakage in preparation. The results of this study indicate that the use of the gentler gravity method provides higher numbers of intact metaphases than the standard rake method. Further, it appears that the use of a high-quality glass slide produces less damage to metaphases than a standard glass slide, which is routinely used for processing the amniotic fluid cell preparations. The smoother glass slide provides a higher number of intact metaphases and a lower number of hypomodal cells.

We conclude that the gravity method of slide preparation and the use of a high-quality Schott glass slide may provide a greater number of intact metaphases. These technical modifications may aid in the interpretation of the karyotype in some instances in which sample size is limited.

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## Circulatory responses to hypovolemia in the pregnant and nonpregnant sheep after pharmacologic sympathectomy

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Circulatory responses to progressive hypovolemia, hypotensive shock, blood reinfusion, and recovery were studied in pregnant and nonpregnant sheep with an intact or pharmacologically ablated sympathetic nervous system produced by administration of 6-hydroxydopamine. These studies also provided an opportunity to assess the contribution of the sympathetic nervous system to the maintenance of vasomotor tone in the pregnant animal at rest. The results show the following: (1) Although there were some differences in the circulatory adjustments to the initial period of blood loss between intact and "sympathectomized" animals, the overall circulatory responses to progressive hypovolemia, shock, blood reinfusion, and recovery were not significantly different in animals with intact or ablated sympathetic nervous systems whether or not they were pregnant. (2) The reasons for the similarity of cardiovascular responses to hypovolemia are the marked increase in catecholamine outputs by the adrenal medulla, which was not affected by 6-hydroxydopamine, and the supersensitivity of the systemic vascular beds of the sympathectomized animal to catecholamines. (3) The contribution of the sympathetic nervous system to the maintenance of the resting vasomotor tone is considerably enhanced during pregnancy, as demonstrated by the chronic effects of adrenergic ablation on the resting arterial pressure. (4) The circulation of the pregnant uterus possesses the ability of autoregulation during chronic changes of perfusing pressure as demonstrated by the differences in the arterial pressure and uteroplacental vascular resistance between intact and sympathectomized animals. (*AM J OBSTET GYNECOL* 1986;154:411-9.)

**Key words:** Sympathectomized sheep, hypovolemia

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It is generally accepted that the sympathetic nervous system is essential for the cardiovascular adjustments to hypovolemia. For instance, the rise in the systemic vascular resistance and the tachycardia that follow blood loss are thought to represent compensatory mechanisms mediated through the sympathetic nervous system to sustain the arterial blood pressure and the cardiac output<sup>1-3</sup> (for a review of the subject see reference 1).

Attempts to investigate the activities of the sympathetic nervous system during blood loss and after blood reinfusion have been made by various investigators.<sup>1-4,7</sup> The results have been conflicting and have depended on variables such as the methods of assessing sympathetic activities and of producing blood loss, animal species and age, anesthesia, and the techniques of recording the cardiovascular functions. Some authors have observed that the surgically "sympathectomized" animals could not tolerate the same volume of rapid blood loss as normal animals; others, who base their opinions on survival rate, believe that no difference exists in the tolerance to hemorrhage between normal and partially sympathectomized animals.<sup>1,4,7</sup>

The results obtained with the use of pharmacologic blocking agents are even more confusing. Some authors have obtained beneficial results with the use of adrenergic-blocking agents such as dibenzylamine in animals experiencing hemorrhage while others observed opposite effects.<sup>6,9</sup>

There is, however, a certain degree of consensus that, in conditions wherein the sympathetic activities are enhanced in the resting state, abolition of these activities would result in poor tolerance to blood loss.<sup>1</sup>

Enhanced sympathetic activities in the maintenance of the resting vasomotor tone, particularly of the capacitance system, have been shown to exist during pregnancy in humans, sheep, and rabbits.<sup>10-12b</sup> These studies, however, were based on the circulatory changes that followed acute interruption of the autonomic nervous impulses either by ganglionic blocking agents or by conduction anesthesia. Whether this enhanced sympathetic tone in the resting state may remain after chronic sympathetic ablation is not known.

We therefore have designed the present studies with the following objectives in mind: (1) to investigate the cardiovascular responses to progressive hypovolemia in pregnant and nonpregnant, unanesthetized sheep in which the  $\alpha$ -adrenergic system has been ablated chronically with 6-hydroxydopamine and (2) to compare the acute and chronic effects of adrenergic ablation in pregnant and nonpregnant animals. The hope behind this particular part of the studies was to find out whether chronic adrenergic ablation would confirm the hypothesis of enhanced sympathetic vasomotor tone

during pregnancy and, if so, what the response to blood loss would be in an animal model with increased sympathetic activities in the resting state.

### Material and methods

A total of 14 pregnant (90 to 120 days) and seven adult (2 to 3 years old) nonpregnant ewes were used for these studies. In the case of the nonpregnant group, each animal served as its own control throughout all of the studies and was subjected to the various experimental periods as described below. The objective of using these nonpregnant ewes was (1) to test the acute and chronic effects of adrenergic ablation under the same experimental conditions and with the use of the same methodology so that comparison with the pregnant group would be more valid and (2) to observe the circulatory response to hypovolemia in the same animal before and after chronic drug-induced sympathectomy.

In the case of the pregnant animals, because of time limitations imposed by the gestational process, the group was divided into the following two subgroups: (1) Control animals (seven ewes) with an intact sympathetic nervous system served to test the circulatory response to progressive hypovolemia in the latter part of ovine gestation and in the presence of a functioning adrenergic system. (2) Sympathectomized animals (seven ewes) served to test the short- and long-term effects of adrenergic ablation in the pregnant sheep as compared with the nonpregnant animal and the cardiovascular response to hypovolemia in nonpregnant animals as compared with that in pregnant ewes with similar gestational ages but with an intact sympathetic system.

### Experimental procedures

**Pregnant animals.** At about 90 to 100 days of gestation, each animal included in the subgroups was chronically instrumented with arterial and venous femoral catheters. The arterial catheter served to monitor the arterial pressure and heart rate as well as to withdraw arterial blood samples when needed for analysis of blood respiratory gases and pH. The venous catheter served for blood withdrawal and reinfusion. To monitor uteroplacental blood flow, the common internal iliac artery was fitted with an electromagnetic flowmeter after ligation of all branches supplying nonuterine structures. The techniques for all of these surgical procedures have been reported elsewhere.<sup>11,13</sup>

**Nonpregnant animals.** In the group of nonpregnant ewes, femoral arterial and venous catheters were implanted by the same techniques used in the pregnant animals; the uterine blood flow was not measured.

In neither the pregnant nor the nonpregnant animals was testing performed before 5 to 7 days of recovery from operation.

**Table I.** Short-term effects of 6-hydroxydopamine in pregnant and nonpregnant sheep

Parameter	Nonpregnant		Pregnant	
	Control	After 6-HD	Control	After 6-HD
Mean arterial pressure (mm hg)	93 ± 3	214 ± 10	83 ± 2	193 ± 23
Heart rate (bpm)	90 ± 9	279 ± 20	101 ± 4	170 ± 65
Uterine blood flow (ml/min)			969 ± 60	555 ± 240

6-HD = 6-Hydroxydopamine.

**Experimental protocol.** The experiments in the pregnant and nonpregnant animals comprised the following steps.

*Collection of control data.* Each animal was brought to a constant temperature room every day at the same time and was held in the standing position in its cage by techniques discussed elsewhere.<sup>11</sup> Resting phasic and mean arterial blood pressure, uteroplacental blood flow (pregnant), and heart rate were recorded continuously for a period of 60 minutes; blood gases and pH were measured once.

*Production of and testing for sympathectomy.* In the pregnant and nonpregnant ewes that were subjected to sympathectomy, after the control data were collected, 6-hydroxydopamine was given intravenously in a dose of 20 mg/kg of body weight, administered during a period of 1 hour. The method of preparing 6-hydroxydopamine for intravenous injections was reported elsewhere.<sup>13</sup> After the injection of 6-hydroxydopamine, blood pressure, uteroplacental blood flow, and heart rate were recorded continuously for the first 5 to 6 hours and once daily for 1 hour thereafter. Blood respiratory gases and pH were analyzed every other day. The effectiveness of sympathetic ablation was ascertained daily by the abolition of the arterial pressor response to a bolus intravenous injection of 200 mg/kg of tyramine.<sup>13</sup> To ensure constant sympathetic ablation, 20 mg/kg of 6-hydroxydopamine was administered intravenously every week for the duration of the study.

*Induction of hypovolemia and blood reinfusion.* In the pregnant and nonpregnant animals, hypovolemia was accomplished by a stepwise reduction of blood volume, which allowed the arterial pressure to set itself at a given level of hypovolemia. We thought this approach would be preferable to Wigger's technique, which aims at a set pressure regardless of the volume of blood withdrawn, because it would better reflect the homeostatic adjusting mechanisms.<sup>1-3</sup> On the appointed day, control values of arterial pressure, heart rate, and uteroplacental blood flow were recorded for 45 to 60 minutes while blood respiratory gases and pH were analyzed once. Thereafter, blood was withdrawn through the femoral venous catheter at a rate of 15 ml/min until 25% of the estimated blood volume had been removed.

The length of the blood withdrawal period varied from 50 to 60 minutes. The hypovolemic period was followed by a period of maximum hypotension (shock) lasting 15 to 20 minutes, during which the circulatory parameters were allowed to stabilize at the levels reached at the completion of the blood withdrawal period. The shed blood was then reinfused at the same rate it was withdrawn. The experiment ended with a recovery period lasting 30 minutes. During all of these periods, the circulatory parameters were recorded continuously while blood respiratory gases and pH were recorded twice.

**Statistical analysis.** The response of each animal (nonpregnant) to blood withdrawal, shock, blood reinfusion, and recovery was related to mean values of that animal obtained during the control period. The responses to hypovolemia of the pregnant ewes with chronic drug-induced sympathectomy were compared to those of the pregnant animals with intact adrenergic systems as well as to the response of the nonpregnant animal to the same procedure. Analysis of variance and *t* test for unpaired and paired values were used to calculate the significance of differences between the group with an intact autonomic nervous system and that with sympathectomy; values of *p* < 0.05 were considered significant.

## Results

**Effects of the surgical procedure.** All animals recovered from the surgical procedure and resumed their normal activities and feeding habits 2 to 3 days after operation. Control baseline values for heart rate, blood pressure, uterine blood flow, and blood respiratory gases and pH were within the range of data previously reported for chronically instrumented animals.<sup>11, 13</sup>

**Short-term circulatory effects of 6-hydroxydopamine.** Table I presents the data on the short-term effects of an intravenous dose of 20 mg/kg of 6-hydroxydopamine. In the control period, the mean arterial pressure of the pregnant group averaged 83 mm Hg; it increased to 193 mm Hg after 6-hydroxydopamine administration (169%). In the nonpregnant animals, control arterial pressure averaged 93 mm Hg and increased to 214 mm Hg (115%) after the injection. Heart

**Table II.** Long-term effects of 6-hydroxydopamine in pregnant and nonpregnant sheep

Parameter	Pregnant		Nonpregnant	
	Intact	Sympathectomy	Intact	Sympathectomy
Mean arterial pressure (mm hg)	80 ± 2	65 ± 2	91 ± 3	86 ± 3
Heart rate (bpm)	108 ± 4	114 ± 2	91 ± 4	92 ± 6
Uterine blood flow (ml/min)	987 ± 60	993 ± 40	—	—
Uterine vascular resistance (mm hg/ml/min)	0.084 ± 0.005	0.067 ± 0.003	—	—

rate also increased markedly in both the pregnant and the nonpregnant animals after 6-hydroxydopamine administration. Blood respiratory gases and pH remained unchanged. In the pregnant animals, uterine blood flow averaged 969 ml/min and decreased to an average of 555 ml/min after 6-hydroxydopamine injection. This marked decrease reflects the intense uterine vasoconstriction produced by catecholamines released from the adrenergic vesicles by 6-hydroxydopamine. These circulatory changes lasted about 3 to 4 hours and the parameters slowly returned to control values within the following 5 hours. These results on the short-term effects of 6-hydroxydopamine were similar to those previously reported.<sup>13</sup>

**Long-term effects of 6-hydroxydopamine.** The test for verification of chronic adrenergic ablation was the disappearance of the pressor response to intravenous injections of 200 mg/kg of tyramine, as described elsewhere.<sup>13</sup> In all pregnant and nonpregnant animals, this pressor response, which averaged 59% in nonpregnant animals and 49% in the pregnant animals, was totally abolished.

In Table II are presented the resting arterial pressure, heart rate, and uteroplacental blood flow both before and after chronic adrenergic ablation.

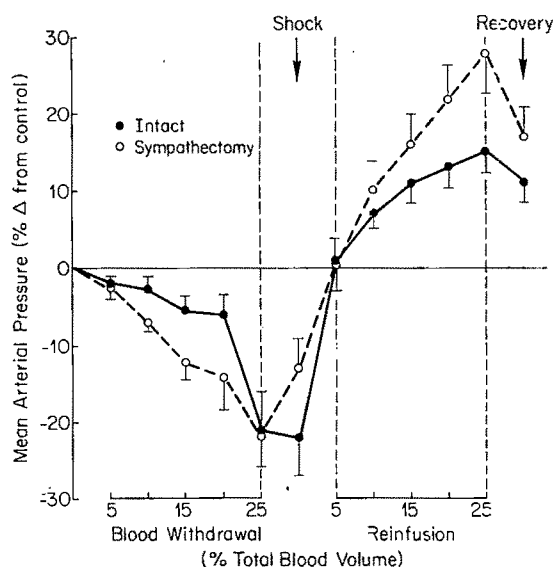
In the control period, the arterial pressure of the nonpregnant animals averaged 91 mm Hg; it decreased to an average of 86 mm Hg during the period of sympathetic ablation and the difference was not significant. The heart rate also did not change after chronic sympathectomy. These results were similar to those previously reported.<sup>13</sup>

In the pregnant animals, the systemic arterial pressure averaged 80 mm Hg in the control period; after chronic sympathectomy, it decreased to an average value of 65 mm Hg and the difference was highly significant ( $p < 0.01$ ). Neither the heart rate nor the uteroplacental blood flow showed any significant alteration during the chronic phase of chemical sympathectomy. The uteroplacental vascular resistance, however, decreased markedly during chronic adrenergic ablation. Blood respiratory gases and pH remained unchanged.

### Hemodynamic response to hypovolemia

**Nonpregnant ewes.** The response of the nonpregnant animals to progressive hypovolemia and blood reinfusion is illustrated in Figs. 1 and 2. The data are presented as percentages of control values because each animal served as its own control and the arterial pressure and heart rate were not significantly different before and after chronic sympathectomy.

**ARTERIAL PRESSURE.** In Fig. 1 are depicted the changes in the mean systemic arterial pressure observed in the nonpregnant animals during progressive blood withdrawal, shock, blood reinfusion, and recovery both before and after chronic adrenergic ablation. During the period of an intact adrenergic system, the arterial pressure before the onset of blood withdrawal averaged 91 mm Hg; after chronic sympathectomy, it averaged 86 mm Hg (Table II). During the blood withdrawal period and in the presence of an intact adrenergic system, the arterial pressure did not begin to change significantly until approximately 20% of the total blood volume had been removed. In contrast, after chronic sympathectomy the arterial blood pressure began to fall after removal of about 10% of the blood volume. If one compares the magnitude of hypotension reached when 15% to 20% of the blood volume had been removed, the difference between the responses of the same group of animals before and after sympathectomy was significant ( $p < 0.05$ ). Thus the nonpregnant sheep with an intact sympathetic nervous system appears to tolerate blood loss up to 20% of its initial blood volume better than the same animal deprived of its sympathetic nervous system (Fig. 1). However, when more blood was withdrawn (25%), the magnitude of the arterial pressure fall in the intact and sympathectomized animals was similar (Fig. 1). These results, which are based on the behavior of the systemic arterial pressure, suggest that the sympathetic nervous system assists in the compensatory mechanisms during the initial period of blood loss. During the period of maximum hypotension (shock) as well as during blood reinfusion and recovery, the responses of the arterial pressure of the intact and sympathectomized animals were not significantly different (Fig. 1).

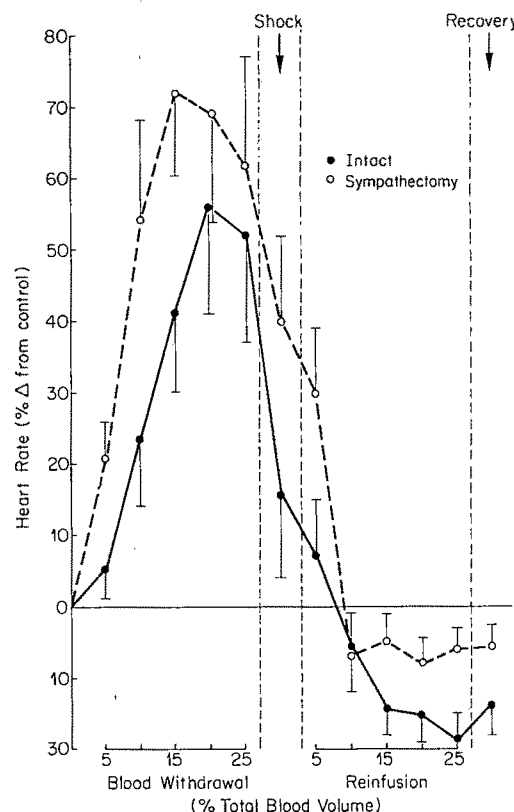


**Fig. 1.** Changes in the systemic arterial pressure of the sympathectomized and intact nonpregnant sheep during blood withdrawal, maximum hypotension, blood reinfusion, and recovery. Values represent mean  $\pm$  1 SE as percentages of changes from control. The difference between intact and sympathectomized animals was not significant.

**HEART RATE.** In Fig. 2 are depicted the changes in heart rate observed in the nonpregnant group before and after sympathectomy. Blood withdrawal of up to 15% of the blood volume produced a progressive increase in the heart rate during both the intact and the sympathectomy periods with a large individual variation, although the difference between the two values was not significant ( $p > 0.05$ ). With further blood withdrawal and during the shock period, the heart rate began to return toward control values in both the intact and sympathectomy experiments. During the reinfusion and recovery periods, the heart rate values were below those observed during the control period in both the sympathectomy and intact experiments; the difference between the two sets of data was not significant ( $p > 0.05$ ). This pattern of heart rate changes during progressive hypovolemia was similar to those previously reported in nonpregnant and pregnant sheep and dogs with intact adrenergic systems.<sup>11, 14</sup>

**Pregnant animals.** The results obtained from the pregnant animals are illustrated in Figs. 3 to 5. The data are presented in absolute values because the control values of arterial pressure and uterine vascular resistance in the chronically sympathectomized pregnant animals were significantly different from those of the intact animals.

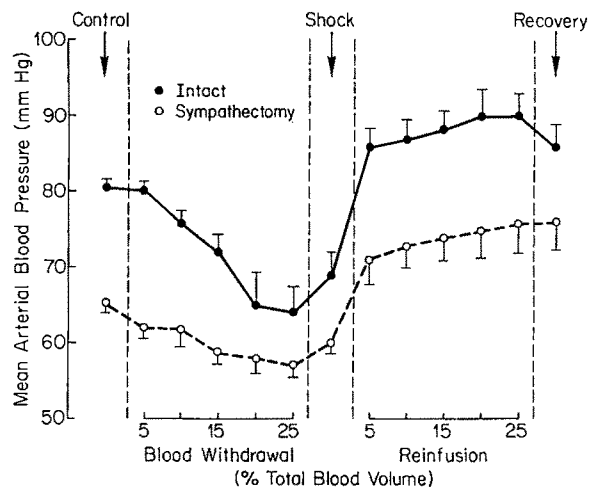
**ARTERIAL PRESSURE.** Before the onset of bleeding, the arterial pressure of the pregnant animals with an intact adrenergic system averaged 30 mm Hg while that



**Fig. 2.** Changes in the heart rate of the sympathectomized and intact nonpregnant sheep during blood withdrawal, maximum hypotension, blood reinfusion, and recovery. Values represent mean  $\pm$  1 SE as percentages of changes from control. The difference between intact and sympathectomized animals was not significant.

of the sympathectomized group was 65 mm Hg (Table II). In Fig. 3 are compared the responses of the mean systemic arterial pressure in the pregnant animals with an intact or an ablated sympathetic nervous system throughout all of the period of the hypovolemic studies. In the group with an intact adrenergic system, withdrawal of 5% of the blood volume produced no changes in the arterial pressure; in the sympathectomized animals, withdrawal of the same volume of blood produced about a 5% fall in the arterial pressure. When 15% of the blood volume was withdrawn, the arterial pressure fell by about 10% in both the intact and sympathectomized groups. Withdrawal of 20% to 25% of the blood volume produced about a 20% decrease in the arterial pressure of the intact animals whereas the fall in the sympathectomized group averaged only 10% ( $p < 0.01$ ). When the slopes of the two curves obtained during the entire period of blood withdrawal were analyzed, the difference between the two groups was significant ( $p < 0.05$ ). Thus the sympathectomized pregnant animal seems to be able to tolerate blood loss some-





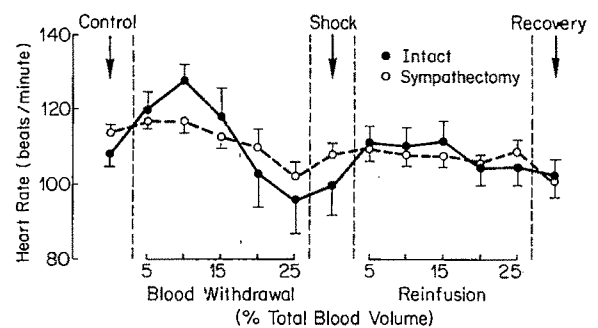
**Fig. 3.** Responses of the systemic arterial pressure of the intact and sympathectomized pregnant sheep to progressive blood withdrawal, maximum hypotension, blood reinfusion, and recovery. The figures (mean  $\pm$  1 SE) are plotted as absolute values. Note the difference in the control pressure of the intact and sympathectomized animals, which demonstrates the enhanced adrenergic vasomotor tone. Note also that for a given degree of hypovolemia, the arterial pressure of the sympathectomized animals fell less than that of the intact animals (for further explanation see text).

what better than the ewe with an intact adrenergic system (Fig. 3).

During the periods of hypovolemic shock, blood reinfusion, and recovery, the behavior of the systemic arterial pressure of the groups with intact and ablated adrenergic systems was similar. Blood respiratory gas and pH values did not change in either group.

**HEART RATE.** In Fig. 4 are compared the heart rate changes of the pregnant animals with intact and ablated adrenergic systems. In the animals with an intact adrenergic system, stepwise blood withdrawal increased the heart rate to a maximum of about 20%, reached when 15% of the blood volume had been removed. When more blood was withdrawn the heart rate began to decline, reaching levels below control with removal of 25% of the blood volume (Fig. 4). In contrast, the heart rate of sympathectomized animals remained at about control values during the period of progressive hypovolemia. During the periods of shock, blood reinfusion, and recovery, the heart rate changes were similar in the intact and sympathectomized animals.

**UTERINE BLOOD FLOW.** Fig. 5 depicts the changes in the uterine blood flow observed in the intact and sympathectomized pregnant animals during the various experimental periods. Before the onset of hypovolemia, uterine blood flow averaged about 950 ml/min and was the same in both groups. The uterine blood flow decreased progressively and in parallel fashion during the various steps of hypovolemia; a maximum decrease of



**Fig. 4.** Response of the heart rate of the intact and sympathectomized pregnant sheep during the periods of hypovolemia, maximum hypotension, blood reinfusion, and recovery. Note the initial increase in heart rate during blood withdrawal; heart rate returned to near control values despite progressive hypovolemia. The heart rate response was greater in the intact animals than in the sympathectomized animals.

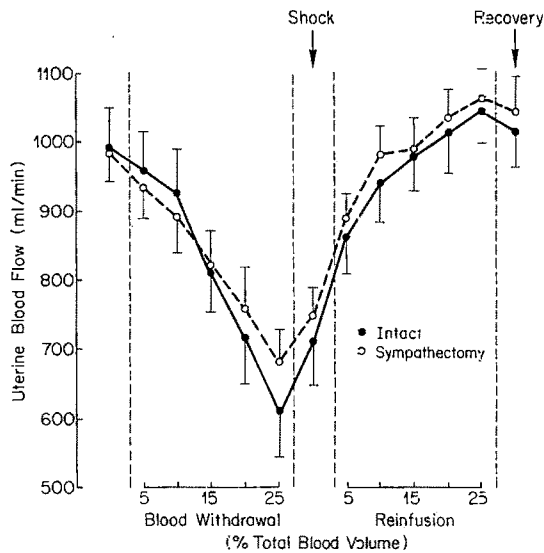
about 35% was observed with removal of 25% of blood volume in the sympathectomized and intact animals. The difference between the slopes of the two groups of animals was not significant. During the shock, reinfusion, and recovery periods the changes in uterine blood flow in the groups with an intact or an ablated autonomic nervous system were similar.

**UTERINE VASCULAR RESISTANCE.** In Fig. 6 are presented the changes in uterine vascular resistance observed in the pregnant group with the sympathetic nervous system intact and after sympathectomy. In the control period before the onset of bleeding, the uteroplacental vascular resistance was significantly higher in the intact animals than in the sympathectomized animals, despite the fact that both groups had about the same uterine blood flow. In both groups the uterine vascular resistance progressively increased in a parallel fashion during the blood withdrawal period; a maximum increase of about 30% occurred when 25% of the blood volume was withdrawn. The resistance slowly decreased toward control values during blood reinfusion and recovery. The difference between the slopes of the two curves was not significant (Fig. 6).

### Comment

The present series of experiments carried out on pregnant and nonpregnant sheep with an intact adrenergic system and after ablation provides a new insight into the role of this system in the maintenance of vasomotor tone and regulation of uteroplacental blood flow in the resting state during pregnancy and in the compensatory mechanisms, which take place during hypovolemia.

**Resting vasomotor tone.** The role of the sympathetic nervous system in the maintenance of vasomotor tone in the nonpregnant condition has been studied exten-

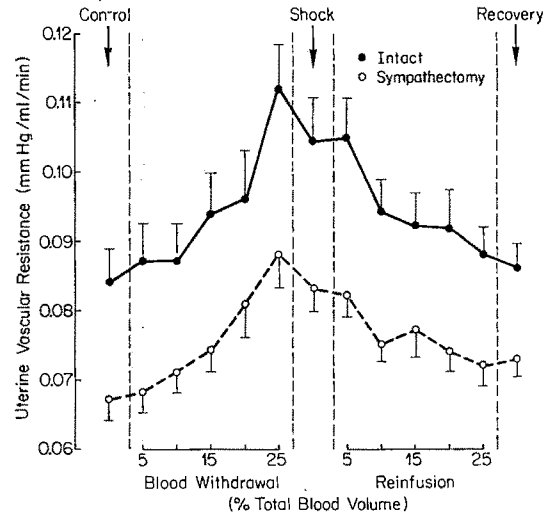


**Fig. 5.** Response of the uteroplacental blood flow of the intact and sympathectomized sheep during progressive hypovolemia, maximum hypotension, blood reinfusion, and recovery. No difference existed between the intact and sympathectomized animals.

sively both in vivo and in vitro (for review see references 1 and 2). The in vivo studies have shown that in the adult animal at rest, the autonomic nervous system exerts a minor role in the regulation of the systemic arterial pressure. Acute or chronic ablation of this system results in insignificant effects on the arterial pressure in humans and animals at rest. Only in response to stress or exercise do the activities of the autonomic nervous system become evident.<sup>1,2</sup>

Our present data on chronic adrenergic ablation add evidence to the previously published work<sup>13</sup> and confirm the minor role of the sympathetic nervous system in hemodynamic regulation in the resting state.

During pregnancy, although the studies have been less extensive, it has been well known for many years that pregnant women undergo a severe hypotension and even circulatory collapse when subjected to any type of conduction anesthesia. Assali et al.<sup>10-15b</sup> carried out systematic studies in which they compared the arterial pressure and cardiac output responses of pregnant and nonpregnant women to ganglionic blocking agents and to high selective spinal anesthesia. Their results showed that, in contrast to nonpregnant subjects, pregnant women exhibited a significant hypotensive response to blockade of the autonomic nervous system; the response increased with the gestational age. On the basis of these studies, these investigators concluded that during human gestation the contribution of the autonomic nervous system to the maintenance of vasomotor tone, particularly in the capacitance sys-



**Fig. 6.** Response of the uteroplacental vascular resistance in the pregnant sheep to progressive hypovolemia, maximum hypotension, and blood reinfusion. During the control period, uteroplacental vascular resistance was significantly lower in the sympathectomized animals than in the intact animals (for explanation see text). Despite the difference, the pattern of changes was the same in the intact and sympathectomized animals.

tem, increases progressively, reaching a peak near term; after delivery, regulation of vasomotor tone reverts to the pattern of nonpregnant subjects.

These results, which have been obtained with short-term inhibition of the autonomic nervous impulses, have been confirmed in recent years in pregnant sheep and rabbits.<sup>10, 13</sup>

The present data obtained from experiments in which the adrenergic system was chronically ablated with 6-hydroxydopamine confirm the above-described studies. They show for the first time that in pregnant sheep at rest the contribution of the sympathetic nervous system to the maintenance of vasomotor tone is considerably enhanced. When this system was ablated chronically, the resting systemic arterial pressure fell significantly, but there was an absence of any hypotension in nonpregnant animals.

Despite the fall in arterial pressure during the chronic phase of adrenergic ablation, the uteroplacental blood flow remained at levels comparable to those observed in animals with an intact sympathetic nervous system. The uteroplacental vascular resistance decreased markedly (Fig. 6). The fall in uteroplacental vascular resistance in the face of diminished arterial pressure during chronic sympathetic ablation indicates that the uteroplacental vascular bed possesses the ability of autoregulation in order to maintain its blood flow at normal levels. Although the presence of autoregulation in the circulation of the pregnant uterus has been suggested in acute conditions,<sup>10</sup> this is the first demonstra-

tion that such a process occurs during chronic sympathectomy.

**Response to hemorrhage.** The evidence cited in the literature in favor of the participation of the sympathetic nervous system in the compensatory cardiovascular adjustments to blood loss is as follows: (1) The decrease in venous return caused by blood loss leads to a decrease in cardiac output and arterial pressure; (2) the fall in the systemic arterial pressure obviously decreases the carotid sinus perfusion pressure, and this latter leads to inhibition of carotid sinus baroreceptors and decreased firing along the sinus nerve; (3) stimulation of the medullary centers and of the thoracolumbar sympathetic chains then ensues, and this produces venous constriction along with an increase in the systemic vascular resistance aimed at supporting the arterial pressure. The sympathetic stimulation together with the vagal inhibition leads to tachycardia, whose aim is to support the cardiac output. All of these mechanisms may be influenced and modified by a variety of experimental conditions including animal species, age, and the status of the animal before blood loss.

Although on theoretical grounds this chain of events seems logical, the experimental evidence obtained in recent years does not lend total support to the above-described mechanisms. For instance, experiments in unanesthetized sheep by us<sup>11</sup> and in unanesthetized dogs by others<sup>14</sup> showed that the  $\beta$ -adrenergic system does not play a significant role in the cardiac changes that follow blood loss. Furthermore, even in unanesthetized animals with an intact sympathetic nervous system, the tachycardia produced by blood loss is not sustained; after a certain degree of hemorrhage, the heart rate starts to decrease and may return to about normal, despite continuation of hypovolemia.<sup>11, 14</sup>

The present data obtained from pregnant and nonpregnant unanesthetized sheep clearly show that the cardiovascular response to blood loss is not appreciably different in the chronically sympathectomized animal compared with the intact animal. In the nonpregnant animals, there was some suggestion that in the absence of the adrenergic system, the animal may not be able to compensate to the initial stage of blood loss as efficiently as when the same animal had an intact adrenergic system. Nevertheless, the overall pattern of changes in heart rate and systemic arterial pressure during hypovolemia, shock, reinfusion, and recovery was practically the same in the intact and sympathectomized states.

In the pregnant animals, some difference existed in the blood pressure and heart rate responses during part of the blood withdrawal period and this difference will be further discussed below. With the overall pattern of

hemodynamic changes, however, it is not possible to detect any significant difference in the behavior of uterine blood flow and uterine vascular resistance as well as in the systemic arterial pressure and heart rate during the period of maximum hypotension, blood reinfusion, and recovery.

The question, then, is what are the factors that permit the sympathectomized animal to adjust to hemorrhage and blood reinfusion in the same manner as the intact animal? Although the answer to this question cannot be accurately given, some hypotheses can be made.

It is well known that blood loss increases greatly the output of catecholamines by the adrenal medulla as well as that of the renin angiotensin system by the kidney.<sup>1, 2</sup> The reports indicate that the output of epinephrine increases fiftyfold to sixtyfold while that of norepinephrine increases tenfold to fifteenfold.<sup>15, 16</sup> These adrenal medullary functions are not affected by the chemical sympathectomy produced with 6-hydroxydopamine.<sup>1, 13-16</sup>

Likewise, it has been convincingly demonstrated that in sympathectomized adult nonpregnant sheep as well as in fetal and neonatal lambs, the cardiovascular system becomes supersensitive to catecholamines.<sup>13</sup> For instance, we have previously shown that, in nonpregnant adult sheep, the arterial pressure response to 0.8  $\mu\text{g/kg}$  of norepinephrine increases from about 25% in intact animals to more than 100% in the sympathectomized condition.<sup>13</sup> Therefore, we believe that the increased production of catecholamines together with the supersensitivity of the systemic vascular beds may account for the similar cardiovascular response to hemorrhage in the intact and sympathectomized animal. The increased production of renin supplements that of catecholamines.

As stated before, despite the similarity of the overall responses to blood loss, there was some difference in the pattern of arterial pressure and heart rate changes in the intact and sympathectomized pregnant animals. The intact pregnant animal seems to have a more rapid hypotension and a greater degree of tachycardia during blood withdrawal when compared with that of the sympathectomized animal. The reason for these differences is not clear. It is possible that the pregnant animal, which has an enhanced sympathetic tone, exhibits a greater degree of supersensitivity to catecholamines than the intact animal. It is also possible that sympathectomy alters vascular permeability and the transfer of fluids from the extravascular to the intravascular compartments during blood withdrawal. This would result in a difference in the effective fluid volume and, consequently, a difference in the degree of tachycardia and fall in blood pressure.

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# Plasma growth hormone concentration in the chronically catheterized ovine fetus during spontaneous term delivery and premature delivery induced by continuous intravascular infusion of low doses of adrenocorticotropin or cortisol to the fetus

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Fetal plasma growth hormone concentrations were measured in 15 pregnant ewes over the last 5 days before delivery. In five chronically catheterized pregnant ewes that underwent spontaneous vaginal delivery  $146 \pm 2$  days (mean  $\pm$  SD) of gestation, fetal plasma growth hormone concentrations fell from  $124.6 \pm 44.1$  ng  $\cdot$  ml $^{-1}$  5 days before delivery to  $35.2 \pm 31.3$  ng  $\cdot$  ml $^{-1}$  at delivery. In three fetuses in which premature delivery was induced by the infusion of cortisol to the fetus at 128 days of gestation, fetal plasma growth hormone levels fell from  $195 \pm 19.1$  ng  $\cdot$  ml $^{-1}$  to  $70.7 \pm 25.5$  ng  $\cdot$  ml $^{-1}$  over the last 5 days of intrauterine life. In seven fetuses in which premature delivery was induced with infusion of synthetic adrenocorticotropin to the fetus beginning at 120 or 130 days of gestation, the fetal plasma growth hormone level did not fall ( $175.6 \pm 75.7$  to  $158.9 \pm 60.1$  ng  $\cdot$  ml $^{-1}$ ). Fetal plasma cortisol concentrations at delivery were significantly higher in the cortisol-infused fetuses ( $214 \pm 38.4$  ng  $\cdot$  ml $^{-1}$ ) than in both control ( $94.4 \pm 33.7$  ng  $\cdot$  ml $^{-1}$ ) and adrenocorticotropin-infused fetuses ( $94.5 \pm 31.9$  ng  $\cdot$  ml $^{-1}$ ). The fall in the fetal plasma growth hormone level in cortisol-induced fetuses may be due to the higher fetal plasma cortisol concentrations achieved in the cortisol-infused compared with the adrenocorticotropin-infused fetuses in which no comparable decrease in growth hormone was observed. These findings suggest that there are significant differences in the fetal response to various experimental regimens used for the induction of premature labor in the sheep. (AM J OBSTET GYNECOL 1986;154:420-3.)

**Key words:** Term delivery, preterm delivery, growth hormone

Plasma growth hormone concentrations in the ovine fetus are elevated in the second half of gestation compared with the values measured in the early neonatal period.<sup>1,2</sup> Although fetal plasma growth hormone concentrations fall to some extent before delivery, the majority of the data available refer to cross-sectional analysis between several fetuses. Few longitudinal data are available in the same fetus over several days during the immediate prepartum period. It is therefore difficult to relate the changes in fetal plasma growth hormone concentration immediately before birth to the other striking changes in different fetal endocrine systems, particularly the adrenal cortex, occurring around the time of delivery.<sup>3-5</sup>

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The aim of the present investigation was to obtain longitudinal data within the same fetus to compare the fall in fetal plasma growth hormone concentrations over the last 5 days preceding spontaneous vaginal delivery at term with the changes produced by the premature induction of labor and delivery following the infusion of cortisol or synthetic adrenocorticotropin continuously to the fetal lamb. The doses of cortisol or adrenocorticotropin infused were chosen to produce elevations in fetal plasma cortisol concentration similar to those observed at normal spontaneous term delivery.<sup>6,7</sup>

## Material and methods

**Animal management and surgical techniques.** Fifteen pregnant Rambouillet-Columbia ewes mated on a single day were used in the study. The day of mating was considered as day 0. Normal term delivery in uncatheterized fetuses in this flock was at  $150 \pm 1$  days' gestation (mean  $\pm$  SD,  $n = 21$ ).<sup>5</sup> Fetal and maternal vascular catheterizations were performed as described in detail previously.<sup>8</sup> Five of the fetuses were infused

with sterile physiologic saline solution (0.9% wt/vol sodium chloride) at a rate of  $0.25 \text{ ml} \cdot \text{hr}^{-1}$ . Premature delivery was induced in three other fetuses following infusion of cortisol hemisuccinate (Efcortelin, Glaxo) at the following rates:  $2.8 \text{ mg} \cdot \text{day}^{-1}$  for 24 hours,  $5.6 \text{ mg} \cdot \text{day}^{-1}$  for the next 24 hours,  $11.2 \text{ mg} \cdot \text{day}^{-1}$  for a further 24 hours, and  $22.4 \text{ mg} \cdot \text{day}^{-1}$  until delivery occurred. Cortisol infusion was commenced at 128 days' gestation. Premature delivery was also induced in the seven remaining fetuses following infusion of  $1 \mu\text{g} \cdot \text{hr}^{-1}$  of synthetic adrenocorticotropin (Synacthen, 1-24 adrenocorticotropin, Ciba) to the fetus. Adrenocorticotropin hormone infusion was commenced at 120 to 122 days' gestation in three fetuses and at 130 to 132 days' gestation in four fetuses.

**Hormone analysis.** Fetal plasma growth hormone<sup>2</sup> and plasma cortisol<sup>9,10</sup> concentrations were measured by radioimmunoassay as previously described.

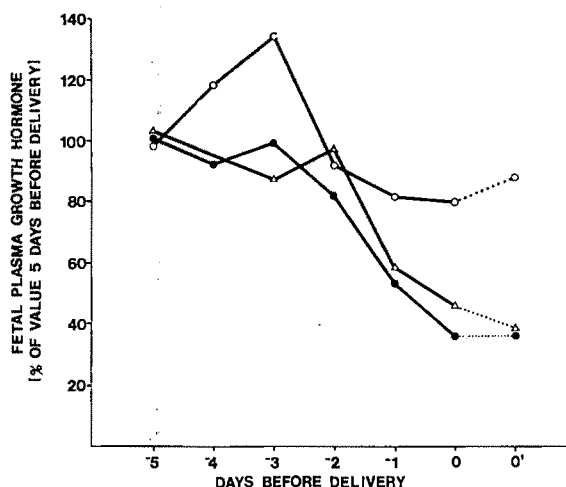
**Data analysis.** Fetal plasma growth hormone concentrations measured over the last 120 hours of intrauterine life in individual control, cortisol-infused, and adrenocorticotropin hormone-infused fetuses were expressed as a percentage of the plasma concentration. Data throughout are given as mean  $\pm$  SD. Statistical analysis was performed on the absolute plasma values by analysis of variance and Student's paired *t* test with the Bonferroni correction for multiple comparisons. Because of the gestational age differences in baseline growth hormone concentrations, the values in Fig. 1 have been plotted as a percentage of the values on the first day of sampling, 5 days before delivery.

## Results

**Outcome of preparations.** All fetuses were delivered vaginally and unassisted. The five chronically catheterized fetuses infused with saline solution only (control group) were delivered alive following spontaneous initiation of parturition at  $145.7 \pm 1.6$  days' gestation. The four fetuses infused with adrenocorticotropin hormone at 130 to 132 days' gestation were delivered alive after  $122 \pm 4.4$  hours of the infusion. The three fetuses infused with adrenocorticotropin hormone at 120 to 122 days of gestation were delivered alive after  $111.9 \pm 2.2$  hours of infusion. For all seven adrenocorticotropin hormone-infused animals, the induction-delivery interval was  $117.7 \pm 3.2$  hours. The three fetuses infused with the escalating dose of cortisol were delivered alive at  $98.8 \pm 7.4$  hours.

### Changes in fetal plasma growth hormone concentration in relation to delivery

**Spontaneous delivery at term of saline-infused fetuses.** Fetal plasma growth hormone fell from a baseline level of  $124.6 \pm 44.1 \text{ ng} \cdot \text{ml}^{-1}$  at 5 days before normal spontaneous term delivery to  $35.2 \pm 31.3 \text{ ng} \cdot \text{ml}^{-1}$  at delivery ( $p < 0.05$ ) (Fig. 1). In the same period the fe-



**Fig. 1.** Percentage fall in fetal plasma growth hormone concentration in five chronically catheterized fetuses infused with saline solution and delivered alive at  $146 \pm 2$  days (mean  $\pm$  SD) of gestation (●—●), in three fetuses delivered alive after  $111.9 \pm 2.2$  hours of infusion with an escalating rate of cortisol administered intravenously to the fetus (△—△), and in seven fetuses delivered alive after  $117.7 \pm 3.2$  hours of synthetic adrenocorticotropin hormone infusion ( $1 \mu\text{g} \cdot \text{h}^{-1}$ ) at either 120 or 130 days' gestation (○—○). The last two samples were taken within the last 12 hours before delivery (O) and at delivery (O').

tal plasma cortisol rose from  $10.1 \pm 6.1 \text{ ng} \cdot \text{ml}^{-1}$  to  $94.4 \pm 33.7 \text{ ng} \cdot \text{ml}^{-1}$  ( $p < 0.05$ ).

**Delivery following infusion of cortisol to the fetus.** Infusion of cortisol at the escalating rate of cortisol administration described also resulted in a significant fall in fetal plasma growth hormone (Fig. 1). The fetal plasma growth hormone concentration fell from  $195 \pm 19.1 \text{ ng} \cdot \text{ml}^{-1}$  to  $70.7 \pm 25.5 \text{ ng} \cdot \text{ml}^{-1}$  ( $p < 0.05$ ). The higher resting concentration in this group compared with the term control fetuses reflects the gestational age difference at the commencement of the study (128 days of gestation compared with 140 days in the saline solution-infused control fetuses at term). Fetal plasma cortisol concentration in this group rose from  $9.8 \pm 5.0 \text{ ng} \cdot \text{ml}^{-1}$  to  $214 \pm 38.4 \text{ ng} \cdot \text{ml}^{-1}$  ( $p < 0.01$ ).

**Delivery following infusion of adrenocorticotropin hormone to the fetus.** In contrast to the other two groups, induction of delivery following adrenocorticotropin hormone infusion to the fetus was not accompanied by a fall in fetal plasma growth hormone levels (Fig. 1). At the beginning of the adrenocorticotropin hormone induction, fetal plasma growth hormone concentration was  $175.6 \pm 75.7 \text{ ng} \cdot \text{ml}^{-1}$ , and at delivery the growth hormone concentration was  $158.9 \pm 60.1 \text{ ng} \cdot \text{ml}^{-1}$ . In the adrenocorticotropin hormone-infused animals the fetal plasma cortisol level rose from  $3.0 \pm 2.1 \text{ ng} \cdot \text{ml}^{-1}$  to  $94.5 \pm 31.9 \text{ ng} \cdot \text{ml}^{-1}$ .

**Comparison between groups.** The mean maximum fetal plasma cortisol concentration achieved in the cortisol-

infused fetuses was greater than in the control fetuses ( $p < 0.003$ ) and the adrenocorticotrophic hormone-infused fetuses ( $p < 0.001$ ). The fetal plasma growth hormone concentrations in the adrenocorticotrophic hormone-infused group at delivery were significantly higher than control or cortisol-infused groups ( $p < 0.05$ ).

### Comment

The resting fetal plasma growth hormone concentrations observed in this study were similar to those previously reported in late gestation.<sup>1,2</sup> The fall in fetal plasma growth hormone level before spontaneous delivery also confirms data produced by ourselves and others.<sup>1,2</sup> The purpose of the present study was to determine whether prematurely induced delivery of the ovine fetus is accompanied by a fall in fetal plasma growth hormone concentration. We have previously demonstrated that cortisol-induced premature delivery mimics the normal term changes in fetal thyrotropin, thyroxine, and triiodothyronine and a fall in reverse triiodothyronine in both the ovine<sup>6</sup> and bovine<sup>11</sup> fetus.

As expected, cortisol-induced delivery produced a fall in plasma growth hormone level similar to that observed at normal term. However, the adrenocorticotrophic hormone-induced deliveries did not produce the normal term fall in growth hormone level. One possible explanation of these unexpected differences between the adrenocorticotrophic hormone induction and cortisol induction may be that at the gestational age studied, the fetal hypothalamic or pituitary mechanisms responsible for the decrease in fetal plasma growth hormone level are less sensitive to cortisol, and thus the fall in fetal plasma growth hormone concentrations only occurs when fetal plasma cortisol is higher than normally present at term. An alternative is that the mechanisms stimulating increased adrenal cortical function at term are not simply dependent on increased secretion of adrenocorticotrophic hormone and other factors may be involved. The significantly higher fetal plasma cortisol concentrations achieved during the cortisol infusion may compensate for the absence of the other changes that occur at normal term delivery, but the lower cortisol levels achieved during the adrenocorticotrophic hormone infusion were inadequate to do this.

A further consideration is that although the adrenocorticotrophic hormone regimen used mimics the absolute rise in cortisol seen at term, it does not replicate the more gradual rise in cortisol levels which commences many days earlier.<sup>5</sup> It may be that exposure to this more gradual rise alters the sensitivity of the hypothalamic pituitary axis to cortisol. It is noteworthy that changes in both fetal and maternal plasma ovine chorionic somatomammotropin concentrations that oc-

cur at normal term also differ from those seen during adrenocorticotrophic hormone-induced premature delivery.<sup>12</sup> The most notable change was an increase in maternal plasma ovine chorionic somatomammotropin during fetal adrenocorticotrophic hormone infusion that was in sharp contrast to the prepartum fall that normally precedes spontaneous vaginal delivery.<sup>12</sup> Thus this earlier study together with our present study demonstrates that changes in certain fetal and maternal hormones that occur during experimentally induced premature delivery are not the same as those that occur at normal parturition.

The mechanism of the preparturient decrease in fetal plasma growth hormone concentration remains unknown. It has been postulated that it is a consequence of the development of inhibitory control of growth hormone release.<sup>13</sup> The fetal pituitary appears relatively insensitive to somatostatin *in vivo* as judged by the only partial suppression achieved by high-dose infusion of somatostatin to the fetal lamb<sup>13</sup> and also by *in vitro* studies of the midgestation human fetal pituitary.<sup>14</sup> Glucocorticoids may act directly, or indirectly through some other induced endocrine change such as the fall in progesterone, to induce an increase in the sensitivity of the somatotrope to somatostatin. Alternatively either the secretion of somatostatin or a growth hormone-releasing factor may be altered.

Studies of cord blood obtained from human infants exposed to betamethasone *in utero* also showed a suppression of growth hormone secretion, particularly in younger infants.<sup>15</sup> Glucocorticoids have previously been shown to suppress growth hormone secretion in adults in response to hypoglycemia,<sup>16-18</sup> but the mechanism underlying this effect has not been clarified.

Our present study demonstrates that the various regimens used to initiate premature delivery in the sheep may mimic the normal term changes in some but not all endocrine variables. Thus extrapolations based on the different prematurely induced parturition models must be viewed with some caution.

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# Influence of sequential doses of 5-hydroxytryptophan on prolactin release

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It is known that the administration of serotonin or its precursors induces the release of prolactin. This study was performed (1) to determine the minimal dose of 5-hydroxytryptophan that would produce a consistent and significant prolactin increase and (2) to establish the frequency of 5-hydroxytryptophan administration necessary to induce a persistent prolactin increase. Nine normal male subjects participated in 27 independent studies following pretreatment with 100 mg of carbidopa given every 8 hours for 2 days. Doses of 0.2, 0.4, and 0.8 mg/kg/hr of 5-hydroxytryptophan were initially infused for 30 minutes, and serum prolactin was measured every 15 minutes for 2½ hours. The urinary 5-hydroxyindoleacetic acid/creatinine ratio was determined in aliquots collected during 3 hours before, during, and after the intravenous infusion. 5-Hydroxytryptophan at a dosage of 0.4 mg/kg/hr was the minimal amount to elicit a consistent and significant prolactin increase ( $p < 0.01$ ). A positive correlation ( $r = 0.907$ ,  $p < 0.002$ ) was also demonstrated between the maximal prolactin response and the 5-hydroxyindoleacetic acid/creatinine ratio. Thus 0.4 mg/kg/hr of 5-hydroxytryptophan was administered sequentially three times at intervals of 2, 4, 6, 8, and 12 hours. With exception of the 12-hour interval a significantly smaller plasma prolactin increase was seen following the third dose of 5-hydroxytryptophan ( $p < 0.05$ ). Furthermore, the nadir for this diminished prolactin response occurred at 4 hours ( $p < 0.01$ ). This phenomenon may represent a down regulation of the serotonin receptors induced by the repetitive administration of 5-hydroxytryptophan. In conclusion, this study has demonstrated a dose-related prolactin response to increasing doses of 5-hydroxytryptophan. The maximum down regulation of prolactin release occurred when 5-hydroxytryptophan was administered at 4-hour intervals. (AM J OBSTET GYNECOL 1986;154:424-7.)

**Key words:** 5-Hydroxytryptophan, prolactin, serotonin

The administration of serotonin or its precursors have been shown to cause a stimulatory effect on prolactin secretion.<sup>1-4</sup> In addition, the prolactin suppression induced by serotonin antagonists following pretreatment with pimozide, a dopamine receptor blocker, gave further evidence for the role of serotonin in the release of prolactin.<sup>5</sup> 5-Hydroxytryptophan, the immediate precursor of serotonin, is commonly used in human studies because it crosses the blood-brain barrier and also because it causes significantly less gastrointestinal side effects than serotonin.<sup>6</sup>

The effects of 5-hydroxytryptophan can be further enhanced by the administration of carbidopa, the peripheral decarboxylase inhibitor. Carbidopa diminishes the peripheral conversion to serotonin, thereby increasing the brain concentration of 5-hydroxytryptophan.<sup>6</sup> It has been recently demonstrated that, following an initial prolactin response to a loading dose of 0.8 mg/

kg/hr of 5-hydroxytryptophan, the same dose given repetitively 2 and 4 hours later resulted in a significant response of plasma growth hormone and cortisol but not of prolactin.<sup>7</sup> This study was carried out to elucidate (1) the minimal dose of 5-hydroxytryptophan that would produce a consistent and significant prolactin response and (2) the sequence of administration of that dose which will be able persistently to elicit a prolactin increase.

## Material and methods

Nine normal male subjects voluntarily participated in completing 27 separate studies. Their ages ranged from 25 to 42 years and their weight from 142 to 183 pounds (within 20% of ideal body weight). All were healthy and were not taking any medication known to affect the levels of prolactin. To determine a dose response of prolactin to increasing intravenous infusions of 5-hydroxytryptophan, three men were initially admitted to the Clinical Research Center on three separate occasions at least 48 hours apart. To diminish the peripheral conversion of 5-hydroxytryptophan to serotonin and also to reduce side effects, 100 mg of carbidopa was administered orally every 8 hours for a total of 6 doses before admission. They were admitted at 7

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AM after an overnight fast and remained in supine position throughout the study. They were not allowed to smoke and were not fed until after the study was completed. All subjects received upon admission a continuous intravenous infusion of 0.9% sodium chloride solution at a rate of 90 ml/hr to insure uniform hydration. Blood samples were obtained through a butterfly needle No. 19 inserted in a vein in the arm not receiving the infusion, which was kept open with a heparin lock. After the intravenous infusion was started, the subjects rested for approximately 2 hours before the study was initiated. After 1000 hours of intravenous infusion, 5-hydroxytryptophan administration was initiated and continued for 30 minutes at a rate of 0.2 mg/kg/hr during the first admission, 0.4 mg/kg/hr during the second admission, and 0.8 mg/kg/hr during the third admission. Blood samples were collected every 15 minutes for 2½ hours, starting 30 minutes before the infusion of 5-hydroxytryptophan. Samples were immediately cooled on ice and centrifuged within 1 hour of collection. Plasma samples were then stored at  $-20^{\circ}\text{C}$  until assayed for prolactin.

Urine was collected at 3-hour intervals before (7 to 10 AM), during (10 AM to 1 PM), and after (1 PM to 4 PM) the infusion of 5-hydroxytryptophan for the determination of 5-hydroxyindoleacetic acid<sup>8</sup> and creatinine (Technicon Autoanalyzer). To establish the sequence of administration of 5-hydroxytryptophan able to elicit a consistent prolactin release, a total of eight normal men (two participated in the first part of the study also) underwent a total of 18 separate studies. Based on the results of the dose-response experiment 0.4 mg/kg/hr was sequentially infused three times for 30 minutes each, at intervals of 2, 4, 6, 8, and 12 hours given on separate days. Carbidopa was also given as described above for 48 hours before admission and 2 hours before each of the 5-hydroxytryptophan infusions. In this part of the study urinary 5-hydroxytryptophan and creatinine were not measured. Hydration, blood sampling, and handling of the specimens were the same as in the first experiment. Plasma concentration of prolactin was measured by a previously reported radioimmunoassay in all samples.<sup>3</sup> The intraassay and interassay coefficient of variation were below 10%. For statistical analysis, one-factor analysis of variance (ANOVA) was performed and the means were compared by multiple-paired *t* test adjusted for multiple comparisons (Bonferroni correction). The area under the curve was calculated by the trapezoidal rule.<sup>13</sup>

## Results

All subjects completed the 27 separate studies without complications. Only one individual experienced transient nausea without vomiting while receiving a 5-hydroxytryptophan infusion of 0.8 mg/kg/hr. Results

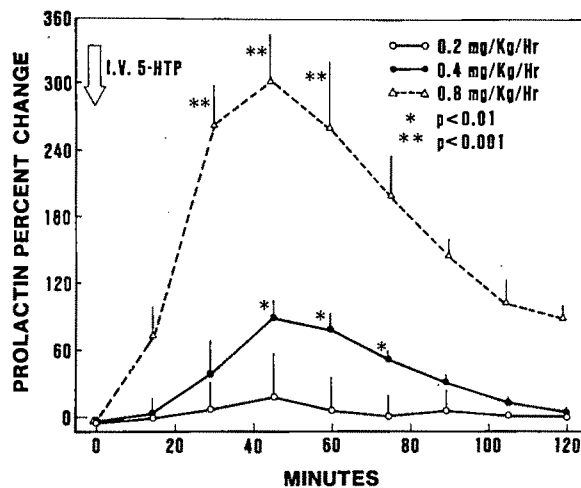


Fig. 1. Plasma prolactin response (mean  $\pm$  SEM) as percent change from baseline, following the administration of increasing doses of 5-hydroxytryptophan (5-HTP). Asterisks represent significance when compared to its own baseline.

of the prolactin response to increasing doses of 5-hydroxytryptophan are shown in Fig. 1. Only one subject receiving the 0.2 mg/kg/hr dose showed some degree of response. Significant and consistent prolactin increases were observed after infusion of 0.4 mg/kg/hr ( $p < 0.01$ ) and 0.8 mg/kg/hr ( $p < 0.001$ ) of 5-hydroxytryptophan. The peak prolactin response and area under the curve to the latter dose was significantly greater than that seen with the former dose ( $p < 0.001$ ). A linear dose response of maximal prolactin increase following the three doses of 5-hydroxytryptophan can be seen in Fig. 2. Furthermore, a positive correlation ( $r = 0.907$ ,  $p < 0.002$ ) was demonstrated between the maximal prolactin response to 5-hydroxytryptophan and the 5-hydroxyindoleacetic acid/creatinine ratio. This ratio increased from  $3.7 \pm 0.8$  to  $4.3 \pm 0.5$  and to  $5.6 \pm 0.9$  in response to the three doses of 5-hydroxytryptophan.

The dose of 0.4 mg/kg/hr of 5-hydroxytryptophan was used in subsequent studies, since it was the smallest dose that elicited a consistent and significant prolactin increase. This dose of 5-hydroxytryptophan, which was infused three consecutive times at intervals of 2, 4, 6, and 8 hours, consistently showed a sequential decrement of prolactin increase. Such blunted prolactin response was not observed when the three doses of 5-hydroxytryptophan were given every 12 hours. The mean ( $\pm$  SEM) prolactin response following the first infusion of 5-hydroxytryptophan at 2 hours ( $771.7 \pm 129$  ng/ml) was statistically different ( $p < 0.01$ ) from the response following the third infusion of 5-hydroxytryptophan at intervals of 2 hours ( $183.3 \pm 140$  ng/ml), 4 hours ( $82.7 \pm 16.6$  ng/ml), 6 hours ( $255 \pm 61$  ng/ml), and 8 hours ( $410 \pm 10$  ng/ml) ( $p < 0.05$ ) (Fig.

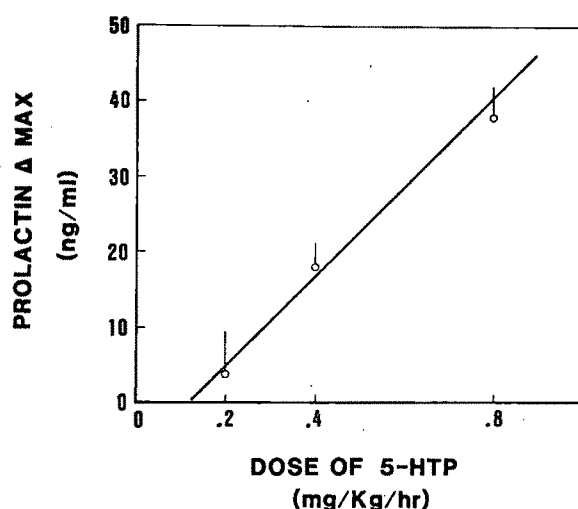


Fig. 2. Linear response of maximal plasma prolactin (mean  $\pm$  SEM) to 0.2, 0.4, and 0.8 mg/kg/hr of 5-hydroxytryptophan.

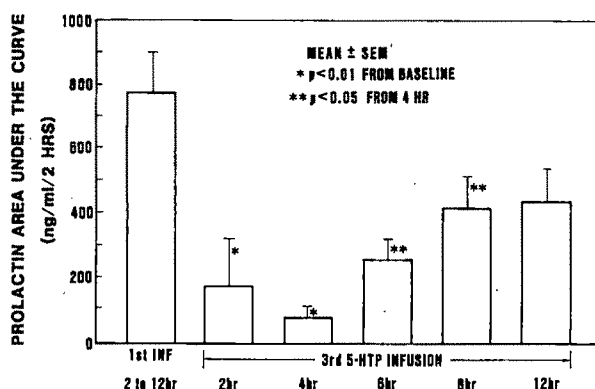


Fig. 3. Combined mean  $\pm$  SEM prolactin area under the curve following the first infusion in the 2- to 12-hour stimulation versus the response obtained after the third infusion in separate experiments performed at 2 to 12 hours. \* = Significantly decreased when compared to baseline of first infusion. \*\* = Significantly increased when compared to 4-hour values.

3). When the data of the three infusions at 2 to 8 hours were combined and compared with those obtained when 5-hydroxytryptophan was infused at 12-hour intervals, a significantly blunted response to the third dose of 5-hydroxytryptophan was again seen ( $p < 0.01$ ) (Fig. 4).

#### Comment

The results obtained in this study indicate that 0.4 mg/kg/hr is the minimal dose of 5-hydroxytryptophan to produce a consistent and significant prolactin response in men. Since 5-hydroxytryptophan crosses the blood brain barrier<sup>6</sup> and 5-hydroxyindoleacetic acid is the principal urinary metabolite of serotonin, the high correlation ( $r = 0.907$ ,  $p < 0.02$ ) between peak prolactin response and the 5-hydroxytryptophan/creatinine ratio further suggest that the increase in serum pro-

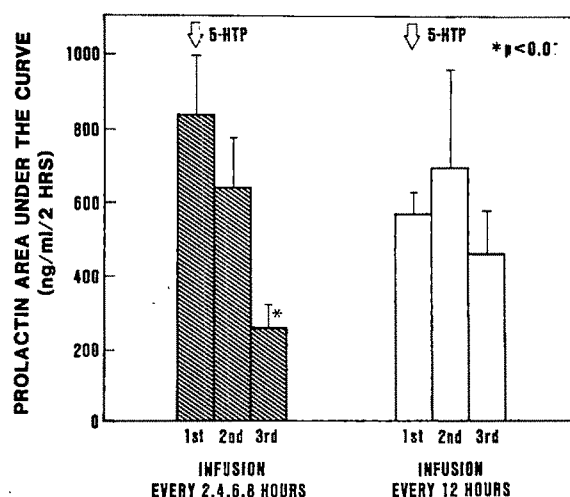


Fig. 4. Combined mean  $\pm$  SEM of prolactin area under the curve at 2 to 8 hours following the first, second, and third infusion of 5-hydroxytryptophan versus responses obtained with infusions given every 12 hours. \* = Significantly lower than first prolactin response.

lactin was probably related to an increase of serotonin in the central nervous system. These results are in agreement with those published in previous studies.<sup>1-7</sup> It was also demonstrated that when 0.4 mg/kg/hr of 5-hydroxytryptophan was given sequentially in three consecutive infusions every 2, 4, 6, 8, and 12 hours, the serum prolactin response between pulses was not the same. Infusions every 2 to 8 hours showed a consistent declining response pattern following the second and third infusion, but significance was only reached following the third dose of 5-hydroxytryptophan. The progressively diminished prolactin response at shorter intervals than every 12 hours may represent a down regulation phenomenon of the serotonin receptors, induced by the repetitive administration of 5-hydroxytryptophan. Thus it can be inferred that endogenous serotonin may regulate its own receptors or at least those responsible for stimulating the release of prolactin. Since it has been previously reported that repetitive administration of 5-hydroxytryptophan results in subsequent release of serum growth hormone and cortisol but not prolactin,<sup>7</sup> it can be suggested that different receptor pathways exist for serotonin stimulation of pituitary hormones. The results of this study cannot clarify whether the 5-HT<sub>1</sub> or 5-HT<sub>2</sub> serotonin receptor population is involved in prolactin release.<sup>11</sup> However, inhibition of serotonin receptor reactivity is not apparent when 5-hydroxytryptophan is administered every 12 hours, which suggests that a complete reestablishment of physiologic status has been achieved. The reported failure in obtaining repetitive prolactin responses with 0.8 mg/kg/hr of 5-hydroxytryptophan given every 2 and 4 hours may be explained by a com-

plete down regulation of the serotonin receptors produced by a dose twice as large the minimal dose found in this study. Alternatively, 5-hydroxytryptophan or serotonin could initially stimulate prolactin release by displacing endogenous dopamine from dopaminergic terminals.<sup>12, 13</sup> It has been suggested that if frequent doses of 5-hydroxytryptophan are given, it could increasingly fail to displace dopamine by a down regulation mechanism and thus the prolactin response would be blunted.<sup>12, 13</sup> However, it has been demonstrated that the concomitant administration of dopamine to rats receiving serotonin did not prevent the rise in serum prolactin.<sup>14</sup> Therefore it can be concluded that 5-hydroxytryptophan, endogenously converted to serotonin, stimulates prolactin release by a hypothalamic mechanism, since serotonin does not stimulate the release of prolactin from in vitro incubations with pituitary glands.<sup>15</sup>

In conclusion, in this study 0.4 mg/kg/hr of 5-hydroxytryptophan was the lowest dose to induce a significant and consistent prolactin response in humans without significant side effects. In addition, repetitive intravenous infusion of that dose of 5-hydroxytryptophan produced a maximum down regulation of prolactin release when given at 4-hour intervals.

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# Fetal $\alpha$ -melanocyte-stimulating hormone levels: No correlation with late fetal growth but increased with diabetes mellitus

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The pars intermedia of the fetal pituitary produces  $\alpha$ -melanocyte-stimulating hormone. Previous reports suggest that  $\alpha$ -melanocyte-stimulating hormone may be a determinant of early fetal growth in animal models. Based on anencephalic fetuses, a similar role was suggested in humans. To examine its relationship to late human fetal growth,  $\alpha$ -melanocyte-stimulating hormone levels were measured by radioimmunoassay in 185 umbilical cord blood samples from anatomically normal fetuses of 28 to 42 weeks' gestation. With use of stepwise multiple regression analysis, no significant relationship was demonstrated between  $\alpha$ -melanocyte-stimulating hormone levels and fetal growth. However, significantly higher levels were found in infants of diabetic mothers ( $p < 0.05$ ) independent of birth weight, gestational age, or both. No significant relationship with other maternal factors related to fetal growth, labor, and delivery was demonstrated. We conclude that fetal plasma  $\alpha$ -melanocyte-stimulating hormone levels do not correlate with late human fetal growth. It is speculated that increased  $\alpha$ -melanocyte-stimulating hormone levels among infants of diabetic mothers might be related to altered neurological maturation. (Am J OBSTET GYNECOL 1986;154:428-30.)

**Key words:**  $\alpha$ -Melanocyte-stimulating hormone, fetal growth, diabetes mellitus

The pituitary gland has long been recognized as an organ important for postnatal growth. In recent years attention has been focused on the human fetal pituitary, which differs from the adult gland by the presence of an intermediate lobe (pars intermedia).<sup>1</sup> The pars intermedia characteristically regresses as the fetus approaches term, which suggests a role specific to the fetal period.<sup>2</sup>  $\alpha$ -Melanocyte-stimulating hormone, one of the polypeptides produced by the pars intermedia, is a member of the proopiomelanocorticotropin family, which consists of adrenocorticotropin hormone,  $\beta$ -lipoprotein,  $\alpha$ -melanocyte-stimulating hormone,  $\beta$ -endorphin, and corticotropin-like intermediate lobe peptide.<sup>3-5</sup>

Previous reports suggest that  $\alpha$ -melanocyte-stimulating hormone is a determinant of fetal growth.  $\alpha$ -Melanocyte-stimulating hormone is the only pituitary peptide known to restore the growth spurt of encephalotomized fetal rats.<sup>6,7</sup> Conversely, the injection of anti- $\alpha$ -melanocyte-stimulating hormone antibodies has inhibited fetal growth in rats.<sup>8,9</sup> Human anencephalic fe-

tuses typically have retarded fetal growth and defective  $\alpha$ -melanocyte-stimulating hormone production, secondary to a hypothalamohypophyseal defect.<sup>10</sup> An effect of  $\alpha$ -melanocyte-stimulating hormone on postnatal growth has not yet been demonstrated.<sup>11</sup>

Previous studies have focused on the acceleratory effect of  $\alpha$ -melanocyte-stimulating hormone on the early pregnancy fetal growth spurt in animal models.<sup>6,9</sup> Human studies have been limited to assaying  $\alpha$ -melanocyte-stimulating hormone, either in anencephalic fetuses or in the pituitaries of human abortuses.<sup>10</sup> The effects of  $\alpha$ -melanocyte-stimulating hormone on continued fetal growth and its production in conditions of normal and/or altered growth have not yet been characterized. The purpose of this study is to examine the relationship between fetal plasma  $\alpha$ -melanocyte-stimulating hormone levels and human fetal growth in the third trimester of pregnancy.

## Material and methods

Umbilical cord samples were collected immediately following the delivery of 185 normal singleton infants at 28 to 42 weeks' gestation, as consecutively as possible during weekdays, including scheduled repeat cesarean deliveries. All deliveries took place at Hutzel Hospital/Wayne State University. Gestational age was assessed by pediatric examination with use of the Ballard score.<sup>12</sup> Infants were classified as small or large for gestational age based on the tenth and ninetieth percentile of body weight (sea level) for gestational age.

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**Table I.** Simple correlations of  $\alpha$ -melanocyte-stimulating hormone with fetal growth

	<i>n</i> (mean $\pm$ SD)	%	<i>r</i> *
Birth weight (gm)	3100 $\pm$ 710		0.04
Birth height (cm)	48.8 $\pm$ 3.7		-0.02
Head circumference (cm)	33.6 $\pm$ 2.31		-0.01
Small for gestational age	27	15	0.08
Large for gestational age	24	13	0.09

\*None of these factors were significantly correlated with  $\alpha$ -melanocyte-stimulating hormone levels.

Specimens were collected in glass tubes containing ethylenediaminetetraacetate, placed immediately on ice, and centrifuged at 4° C. The supernatant (plasma) was stored in tightly closed plastic tubes at -20° C until analysis was performed. The method used for analysis is a modification of the technique of Orth, Tanaca, and Nicholson.<sup>13</sup> Briefly, this method consists of plasma extraction, radioimmunoassay with use of a sensitive and specific  $\alpha$ -melanocyte-stimulating hormone antiserum (Bio Products No. 1660-001, lot No. 203), a delayed tracer purification (QUSO methoc), and a second antibody (goat antirabbit  $\gamma$ -globulin, Hazelton Research Products). This assay has a sensitivity of 5 to 10 pg/ml with a very minimal cross-reaction to other propriomelanocorticotropin peptides (adrenocorticotrophic hormone 1-17 (5.4%), 1-39 (1.2%), 1-16 (.03%), and the remainder <0.05%).

The relationship of  $\alpha$ -melanocyte-stimulating hormone to fetal growth was analyzed with use of linear and multiple regression with  $p < .05$  considered significant. For simplicity in computation, cord plasma  $\alpha$ -melanocyte-stimulating hormone level was considered the outcome variable. Explanatory variables representing fetal growth included birth weight, birth weight adjusted for gestational age, infants small or large for gestational age, the length and head circumference of the neonate. Potentially confounding factors related to fetal growth and/or neuroendocrine function were also considered in the analysis. These included gestational age, sex, race, multiparity, premature rupture of membranes, drug dependence, hypertension, diabetes mellitus, presence of labor, duration of labor, and cesarean birth.

### Results

The correlation of cord plasma  $\alpha$ -melanocyte-stimulating hormone levels with parameters of fetal growth are shown in Table I. No significant relationship between these hormone levels and fetal growth was apparent. Adjusting  $\alpha$ -melanocyte-stimulating hormone levels for gestational age with use of multiple regression failed to alter this finding.

The correlations of fetal  $\alpha$ -melanocyte-stimulating hormone levels with factors related to fetal growth and/

**Table II.** Simple correlations of  $\alpha$ -melanocyte-stimulating hormone related to fetal growth and/or neuroendocrine function

	<i>n</i> (mean $\pm$ SD)	%	<i>r</i>
Gestational age (wk)	39 $\pm$ 2.5		0.04
No. of infants	185		
Male infants	40	49	-0.01
Black infants	141	76	0.03
Multiparity	116	63	0.03
Premature rupture of membranes	19	10	0.05
Drug dependence	9	5	0.00
Hypertension	11	6	0.01
Diabetes mellitus	13	7	0.16*
Presence of labor	156	85	-0.06
Duration of labor (hr)	8.1 $\pm$ 6.9		-0.05
Cesarean birth	69	37	0.12

\* $p < 0.05$ .

or neuroendocrine function are reported in Table II. Infants of diabetic mothers had significantly higher  $\alpha$ -melanocyte-stimulating hormone levels ( $8.4 \pm 2.1$  pg/ml) than infants of nondiabetic mothers ( $7.2 \pm 1.9$  pg/ml), which accounted for 2.5% of the variance of fetal  $\alpha$ -melanocyte-stimulating hormone. This finding was unaffected by adjustment for gestational age, birth weight, or birth weight for gestational age with use of multiple regression. The 37% cesarean rate in this study was much higher than the overall cesarean rate in our institution.

### Comment

The major finding of our study was that fetal plasma  $\alpha$ -melanocyte-stimulating hormone levels do not correlate with late fetal growth. A second, unanticipated finding was the increased  $\alpha$ -melanocyte-stimulating hormone levels found among infants of diabetic mothers. Both findings merit further consideration.

This study differs from those previously reported,<sup>6, 10</sup> in that it has focused on human pregnancies in the third trimester, a period at which major alterations of human fetal growth occur. Previous studies have focused on the relationship of  $\alpha$ -melanocyte-stimulating hormone to fetal growth during early gestation in animals. There is no simple way to explain the discrepancy between our study and previous studies suggesting a major role of  $\alpha$ -melanocyte-stimulating hormone in early fetal growth. Although this discrepancy can be attributed to functional variations related to differences in species or stage of gestation or both, we cannot disclaim a possible role in late human fetal growth just on the basis of measuring  $\alpha$ -melanocyte-stimulating hormone levels. The effect of a hormone depends on its rates of production, destruction and excretion, and receptor sensitivity. In addition, more specific studies of production utilization and metabo-

lites will now be necessary to define such possible variables and their effects, if any, on growth.

The finding of increased  $\alpha$ -melanocyte-stimulating hormone levels among infants of diabetic mothers must be interpreted very cautiously, since it was a small effect and was unanticipated. It could represent nothing more than a statistical "fluke" related to the multiple comparisons made in this study. There are, however, several theoretical possibilities that might explain an association of diabetes with increased  $\alpha$ -melanocyte-stimulating hormone levels. Delayed neurological maturation among infants of diabetic mothers has been suggested previously.<sup>14</sup>  $\alpha$ -Melanocyte-stimulating hormone levels among these infants might reflect a delay in the regression of the pars intermedia of fetal pituitary concomitant with delayed neurologic maturation. Another potential explanation is based on the need for an intact fetal pituitary gland in order for the fetal pancreas to respond to hyperglycemia.<sup>15</sup> The pars intermedia produces a factor that cross-reacts with adrenocorticotrophic hormone, promoting insulin secretion in obese hyperglycemic mice.<sup>16</sup> A third speculation is based on the finding of a substance related to  $\alpha$ -melanocyte-stimulating hormone in placental extracts.<sup>17</sup> The increase of  $\alpha$ -melanocyte-stimulating hormone among infants of diabetic mothers might be the result of the contribution of the large diabetic placenta.

In summary, although  $\alpha$ -melanocyte-stimulating hormone may be an important regulator of early fetal growth in animals, it does not appear that its levels in fetal plasma correlate with late human fetal growth. The relationship of fetal  $\alpha$ -melanocyte-stimulating hormone with maternal diabetes mellitus merits further research.

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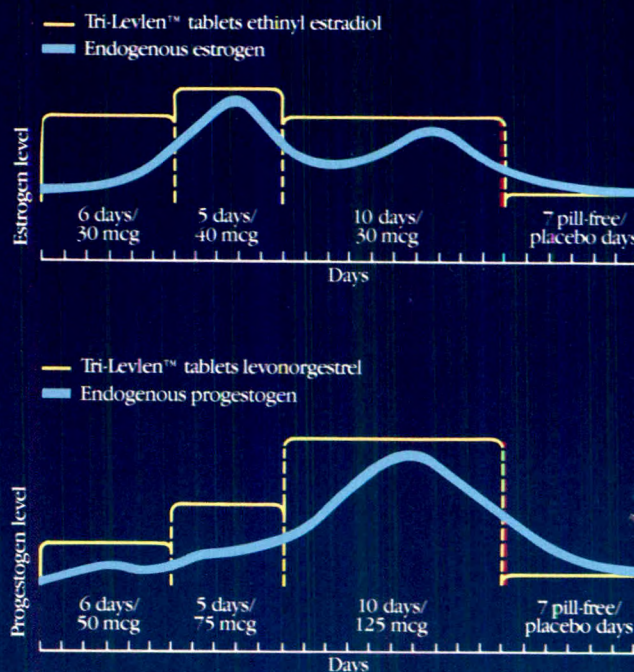
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ious as well as minor side effects have been reported following the use of all oral contraceptives. These include thromboembolic disease. The physician should remain alert to the earliest manifestations of any symptom of serious disease and discontinue oral contraceptive therapy when appropriate. These data represent only the results of selected studies. Therefore, before prescribing, the physician should read and remain alert to data found in the complete prescribing information for the product. Please also see brief summary of prescribing information on the last page of advertisement.

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## BRIEF SUMMARY

**Tri-Levlen™**—6 brown tablets, each containing 0.050 mg of levonorgestrel (dl-13 beta-ethyl-17-alpha-ethinyl-17-beta-hydroxygon-4-en-3-one), a totally synthetic progestogen, and 0.030 mg of ethinyl estradiol (19-nor-17 $\alpha$ -pregna-1,3,5(10)-trien-20-yne-3,17-diol), 5 white tablets, each containing 0.075 mg levonorgestrel and 0.040 mg ethinyl estradiol; 10 light-yellow tablets, each containing 0.025 mg levonorgestrel and 0.030 mg ethinyl estradiol (7 light-green tablets containing inert ingredients are included in the 28-day triphasic regimen).

**Indications and Usage**—Tri-Levlen Tablets are indicated for the prevention of pregnancy in women who elect to use oral contraceptive (OCs) as a method of contraception.

**Contraindications**—OCs should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep-vein thrombophlebitis or thromboembolic disorders. 3. Cerebral-vascular or coronary artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Known or suspected pregnancy (see Warning No. 5). 8. Benign or malignant liver tumor which developed during the use of OCs or other estrogen-containing products.

## Warnings

**Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.**

The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. **Thromboembolic Disorders and Other Vascular Problems.** An increased risk of thromboembolic and thrombotic disease associated with the use of OCs is well established. Three principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OCs are 1.5 to 2 times more likely than nonusers to develop these diseases without evident cause.

**CEREBROVASCULAR DISORDERS**—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

**MYOCARDIAL INFARCTION**—An increased risk of myocardial infarction associated with the use of OCs has been reported, confirming a previously suspected association. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of pre-eclamptic toxemia) the higher the risk of developing myocardial infarction, regardless of whether the patient was an OC user or not. OCs, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified, it is quite likely that the same synergistic action exists, but perhaps to a lesser extent.

**RISK OF DISEASE**—In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism, including coronary thrombosis, is directly related to the dose of estrogen used in OCs. Preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the United States.

**ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES**—A large prospective study carried out in the U.K. estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OCs according to age, smoking habits and duration of use. The overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000) the risk being concentrated in older women, in those with a long duration of use and in cigarette smokers. It was not possible, however, to examine the interrelationships of age, smoking and duration of use, nor to compare the effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. The available data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. The estimates of risk of death for each method include the combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OCs) plus the risk attributable to pregnancy or abortion in the case of the natural family. This latter risk varies with the effectiveness of the contraceptive method. The study method, that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OCs in women over 40 who smoke. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with OCs increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes or history of pre-eclamptic toxemia and, especially, by cigarette smoking. The physician and the patient should be alert to the earliest manifestations of thrombophlebitis and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four- to six-fold increased risk of postsurgery thromboembolic complications has been reported in oral contraceptive users. If feasible, OCs should be discontinued at least 4 weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization.

**PERSISTENCE OF RISK OF VASCULAR DISORDERS**—Findings from one study in Great Britain involving cerebrovascular disease and another study in the United States concerning myocardial infarction suggest that an increased risk of these conditions in users of OCs persists after discontinuation of the OC. In the British study, the risk of cerebrovascular disease remained elevated in former OC users for at least six years after discontinuation. In the U.S. study, an increased risk of myocardial infarction persisted for at least 9 years in women 40- to 49-years-old who had used OCs for five or more years. The findings in both these studies require confirmation since they are inconsistent with other published information.

2. **Ocular Lesions.** There have been reports of neuro-ocular lesions, such as optic neuritis or retinal thrombosis, associated with the use of OCs. Discontinue OC medication if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. **Carcinoma.** Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina and liver. Certain synthetic progestogens, now currently contained in OCs, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case-control studies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the cases found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time OCs were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs. Several studies have found no increase in breast cancer in women taking OCs or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with OCs, found an excess risk in the subgroups of OC users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of OCs has been well-documented. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with OCs. Close clinical surveillance of all women taking OCs is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use OCs.

4. **Hepatic Tumors.** Benign hepatic adenomas have been found to be associated with the use of OCs. One study showed that OC formulations with high hormonal potency were associated with a higher risk than lower potency formulations. Although benign hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of OCs. Two studies relate risk with duration of use of OCs, the risk being much greater after 4 or more years of OC use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women taking OCs. The relationship of these drugs to this type of malignancy is not known at this time.

5. **Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring and Malignancy in Female Offspring.** The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence at the present time that OCs further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OCs. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including OCs, in pregnancy. One case-control study has estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triphasic and possibly other types of polyploid are increased among abortions from women who become pregnant soon after stopping OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortions of pregnancies conceived soon after stopping OCs is unknown. It is recommended that, for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed

period and further use of OCs should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue OCs with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no firm information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. **Gallbladder Disease.** Studies report an increased risk of surgically confirmed gallbladder disease in users of OCs and estrogen. One study, an increased risk appeared after 2 years of use and doubled after 4 or 5 years of use. In one of the other studies increased risk was apparent between 6 and 12 months of use.

7. **Carbohydrate and Lipid Metabolism.** Effects. A decrease in glucose tolerance has been observed in a significant percent of patients on OCs. For this reason, prediabetic and diabetic patients should be carefully observed while receiving OCs. An increase in triglycerides and total phospholipids has been observed in patients receiving OCs. Three studies have been performed with Tri-Levlen Tablets (Levonorgestrel and Ethinyl Estradiol Tablets Triphasic Regimen) formulation and no significant alterations in metabolism were noted, with the exception of a slight increase in triglyceride levels in one study. The clinical significance of findings remains to be defined.

8. **Elevated Blood Pressure.** An increase in blood pressure has been reported in patients receiving OCs. In some women, hypertension may occur within a few months of beginning OC use. In the first year of use, the prevalence of women with hypertension is low and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer use and in the fifth year of use is two- and a-half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given OCs. Hypertension that develops as a result of taking OCs usually returns to normal after discontinuing the drug.

9. **Headache.** The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent or severe, requires discontinuation of OCs and evaluation of the cause.

10. **Bleeding Irregularities.** Breakthrough bleeding, spotting and amenorrhea are frequent reasons for patients discontinuing OCs. Breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. Undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Change to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of OCs. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. **Ectopic Pregnancy.** Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. **Breast-feeding.** OCs given in the postpartum period may interfere with lactation. There may be a decrease in the quantity quality of the breast milk. Furthermore, a small fraction of the hormonal agents in OCs has been identified in the milk of nursing mothers. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of OCs should be deferred until the infant has been weaned.

**Precautions**—**GENERAL.** 1. A complete medical and family history should be taken prior to initiation of OCs. The pretest and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic or including Papanicolaou smear and relevant laboratory tests. As a general rule, OCs should not be prescribed for longer than necessary without another physical examination and PAP smear being performed. 2. Under the influence of estrogen-progestogen preparations preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed. The drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking OCs should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. OCs may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which may be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice receiving OC therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Sex hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in patients. 7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. Clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by OC therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, possible that if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of OC therapy when relevant specimens submitted. 10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OCs. Increased sulphydrylphthalate retention. b. Increased prothrombin and factors VII, VIII, IX and X. decreased antithrombin III. increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or T4 by radioimmunoassay. Free T3 resin uptake decreased, reflecting the elevated TBG. Free T4 concentration is unaltered. d. Decreased pregnandiol excretion. e. Reduced response to metoprolol test.

**Information for the Patient**—See Patient Package Leaflet.

**Drug Interactions**—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline. **Carcinogenesis**—See "Warnings" section for information on the carcinogenic potential of OCs.

**Pregnancy**—Pregnancy Category X. See "Contraindications" and "Warnings."

**Nursing Mothers**—See "Warnings."

**Adverse Reaction**—An increased risk of the following serious adverse reactions has been associated with the use of OCs: "Warnings": thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertensive gallbladder disease, benign hepatomas, congenital anomalies.

There is evidence of an association between the following conditions and the use of OCs, although additional confirmatory study needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients receiving OCs and are believed to be drug-related: Nausea or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first 3 months of use. Other reactions, as a general rule, are seen much less frequently or only occasionally: gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding, spotting, change in menstrual flow, dysmenorrhea, amenorrhea during and after treatment, temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes (tenderness, enlargement and secretion, change in cervical erosion and cervical secretion; possible diminution in lactation when immediately postpartum; cholestatic jaundice, migraine, increase in size of uterine leiomyomata, rash (allergic), mental depression, reduced tolerance to carbohydrates, vaginal candidiasis, change in corneal curvature (steepening); intolerance to contact lens. The following adverse reactions have been reported in users of OCs, and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, dizziness, nervousness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, hemolytic uremic syndrome.

**Acute Overdose**—Serious ill effects have not been reported following acute ingestion of large doses of OCs by young children. Overdose may cause nausea, and withdrawal bleeding may occur in females.

**Dosage and Administration**—To achieve maximum contraceptive effectiveness, Tri-Levlen Tablets (Levonorgestrel and Ethinyl Estradiol Tablets—Triphasic Regimen) must be taken exactly as directed and at intervals not exceeding 24 hours. (If Tri-Levlen is first taken later than the first day of the first menstrual cycle of medication or postpartum, contraceptive reliance should not be placed on it after the first 7 consecutive days of administration. The possibility of ovulation and conception prior to initiation of medication should be considered.) Any time the patient misses 1 or 2 brown, white or light-yellow tablets, she should also use another method of contraception until she has taken a tablet daily for 7 consecutive days.

For full details on dosage and administration see prescribing information and package insert.

**References:** 1. *FDA Drug Bull* 1984;14(1):2-3. 2. Data submitted to FDA. 3. Adapted from Briggs MH, in Brotons I (ed). *New Considerations in Oral Contraception*. Proceedings of an International Symposium. Leuven, Belgium (1981). 1982. pp 131-151. 4. Data on file. Berlex Laboratories Cumulative Data Base. 1982-1985.



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# Pergolide and bromocriptine for the treatment of patients with hyperprolactinemia

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A prospective study of 22 women with hyperprolactinemia from various causes was performed with use of bromocriptine in nine patients and pergolide in 13 patients. The administration of 50  $\mu$ g of pergolide followed by 100  $\mu$ g on the second day showed significant decrements ( $p < 0.01$ ) in systolic and diastolic blood pressure in either standing or lying position. However, 25  $\mu$ g of pergolide followed by 50  $\mu$ g did not lower blood pressure. Both 25 and 50  $\mu$ g of pergolide induced a maximal and significant ( $p < 0.005$ ) inhibition of prolactin at 8 hours and remained suppressed for at least 24 hours. Long-term treatment with either bromocriptine or pergolide was continued for 48 weeks. Both dopamine agonists demonstrated a similar degree of prolactin inhibition throughout time. However, only patients treated with pergolide had higher levels ( $p < 0.05$ ) of luteinizing hormone and follicle-stimulating hormone. Resumption of spontaneous menses and cessation of galactorrhea occurred at similar times in both groups. It can be concluded that either dopamine agonist can be safely given to patients with hyperprolactinemia. (AM J OBSTET GYNECOL 1986;154:431-5.)

**Key words:** Hyperprolactinemia, dopamine agonists

Dopamine and dopamine agonists are known to be potent inhibitors of prolactin secretion. In the past 10 years ergot alkaloids and especially bromocriptine mesylate have been effectively used to restore regular menses and to reduce the size of a microprolactinoma in patients with hyperprolactinemia.<sup>1</sup> In vivo and in vitro studies have also demonstrated that bromocriptine has a deleterious effect on human and rat pituitary adenomas but not on normal rat pituitary tissue.<sup>2,3</sup> However, the use of bromocriptine is not devoid of side effects and is usually administered in divided daily doses to reduce side effects and to obtain a more uniform concentration of the drug in blood. Recently pergolide mesylate, a new ergot derivative with apparently greater potency on a weight-per-weight basis and one that can be administered once a day, has been developed.<sup>4,5</sup> Human and animal studies have revealed that a single dose of this dopamine agonist has a potent inhibitory effect on prolactin release, which lasted for at least 24 hours.<sup>6</sup>

The aim of the present study was to determine (1) the safety and efficacy of pergolide mesylate during the initial 48 hours of treatment and (2) to compare its

effectiveness with bromocriptine in patients with hyperprolactinemia and menstrual dysfunction with or without prolactin-secreting pituitary adenoma.

## Material and methods

A total of 22 women with amenorrhea, galactorrhea, and hyperprolactinemia agreed to participate in the study. Of these, 13 women ( $30.5 \pm 1.0$  years of age and  $136 \pm 8.1$  pounds) were treated with pergolide, and the remaining nine patients ( $28.4 \pm 1.8$  years of age and  $132.4 \pm 7.1$  pounds) received bromocriptine. Of the 13 patients treated with pergolide, nine received an initial dose of 50  $\mu$ g the first day and 100  $\mu$ g the second day of treatment. This dose was increased to a maximum of 300  $\mu$ g daily only if serum prolactin remained  $>20$  ng/ml. These nine patients were randomized with the nine women receiving bromocriptine. The remaining four patients were not randomized and received an initial dose of 25  $\mu$ g on the first day, 50  $\mu$ g the second day, and a further increased dose 1 week later only if necessary and if the medication was tolerated. All daily doses of pergolide were administered in a single dose immediately after dinner. Bromocriptine was given as previously reported, starting with 2.5 mg daily after dinner and increasing thereafter to a maximum of 7.5 mg as tolerated and if serum prolactin remained  $>20$  ng/ml.<sup>7</sup> Before initiation of treatment all patients underwent ancillary blood and urine tests, a computerized tomographic scan or polytomography of the sella turcica, plasma measurement of prolactin, luteinizing hormone, follicle-stimulating hormone, thyrotropin, growth hormone, and cortisol. To determine

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**Table I.** Clinical and laboratory baseline data (mean  $\pm$  SEM)

Treatment	No.	Amenorrhea	Galactorrhea	Adenoma		Prolactin (ng/ml)*	Growth hormone (ng/ml)†
				Micro	Macro		
Bromocriptine	9	9	9	3	—	153 $\pm$ 47	1.9 $\pm$ 0.5
Pergolide	13	13	13	2	2	232 $\pm$ 85	2.7 $\pm$ 1

\*8-20.

†0.3-7.5.

‡4.0-20.

§3.2-9.0.

||1.0-6.0.

the patient's estrogen status they all received an intramuscular injection of 100 mg of progesterone in oil.<sup>8</sup> Six of the nine women receiving bromocriptine and nine of the 13 receiving pergolide had a vaginal bleeding response to progesterone. Only patients receiving pergolide were admitted to the Clinical Research Center for 48 hours following the initiation of treatment. They were admitted at 5 PM, and a No. 19 butterfly needle was inserted in an arm vein and kept open with a heparin lock. Blood samples were taken before pergolide administration and every hour thereafter for the first 6 hours, followed by sampling every 2 hours until the end of the 48 hours. Both erect and supine blood pressures were determined twice at 1-hour intervals and 1, 3, 8, and 24 hours after the first and second dose of pergolide. All patients had a low-fat diet at the usual mealtime and were allowed to ambulate and keep their usual 8-hour night sleep schedule. At the end of the 2 days they were discharged and their progress followed, as were the patients receiving bromocriptine, at 1, 2, and 4 weeks thereafter for up to 48 weeks. In each visit vital signs were noted and blood samples taken. In all blood samples plasma luteinizing hormone, follicle-stimulating hormone, and prolactin levels were determined by previously reported radioimmunoassay.<sup>8,9</sup> All medications were discontinued if pregnancy ensued or if significant side effects occurred. Statistical analysis of data obtained during the initial 48 hours of pergolide treatment was performed by repeated measures analysis of variance. Whenever significance was determined, the multiple-paired *t* test was used to establish the points of significance from baseline. *P* value was adjusted by the Bonferroni correction for multiple comparisons to an overall significance level of 0.05.<sup>10</sup> Comparisons between results of pergolide and bromocriptine were performed by Student's *t* test. Analysis of side effects was performed by  $\chi^2$ .

## Results

There were no significant differences in clinical or baseline laboratory values between patients receiving either dopamine agonist (Table I). The initial 48-hour

in-hospital evaluation of the safety of pergolide revealed that a dose of 50  $\mu$ g on the first day resulted in significant decrements of both systolic ( $p < 0.02$ ) and diastolic ( $p < 0.05$ ) blood pressure in either standing or supine position at 8 hours. At 24 hours there was a significant lowering of the systolic blood pressure only, in both standing and supine positions ( $p < 0.02$ ). The administration of 100  $\mu$ g on the second day resulted in significant hypotension ( $p < 0.02$ ) at 8 hours of diastolic blood pressure only in both standing and supine positions (Figs. 1 and 2). This was accompanied by dizziness, light-headedness, nausea, and sometimes vomiting at the time of blood pressure measurement in the standing position. These symptoms caused by the lowering of blood pressure were short-lived, since no abnormalities were detected throughout the year of continued use. Because of the significant suppression of blood pressure in patients receiving 50 and 100  $\mu$ g of pergolide, four patients were treated with 25  $\mu$ g followed by 50  $\mu$ g on the second day. Three of the four patients participated in the acute and long-term treatment and the other in the long-term study only. As a group there were no significant changes in blood pressure; however, one of these patients demonstrated a 25% decrease in standing systolic and diastolic blood pressure accompanied by nausea and dizziness. Although patients receiving bromocriptine did not have blood pressure measured during the first 48 hours of treatment, no significant changes were apparent during the long-term use.

The immediate effect of pergolide on plasma prolactin release is depicted in Fig. 3. The initial dose of 50  $\mu$ g induced a rapid and significant inhibition of 30%  $\pm$  2% (mean  $\pm$  SEM) at 2 hours ( $p < 0.03$ ) with a maximum of about 80% reduction at 8 hours. This was followed by a plateau for the remainder of the day ( $p < 0.0005$ ). The administration of the second dose (100  $\mu$ g) of pergolide resulted in an additional 60% reduction ( $p < 0.0005$ ) and a prolonged second plateau. By the eighth hour of the second day all of these nine patients had plasma prolactin concentration of  $< 20$  ng/ml. The patients who were initiated on 25  $\mu$ g

Luteinizing hormone (mIU/ml)‡	Follicle-stimulating hormone (mIU/ml)§	Thyrotropin (uU/ml)
5.6 ± 1.3	7.0 ± 2.2	2.6 ± 0.4
7.9 ± 1.0	6.0 ± 0.9	3.4 ± 0.6

of pergolide showed a similar pattern of prolactin release not significantly different than that observed with 50 µg. However, the initial significant suppression occurred at 8 hours ( $p < 0.025$ ) instead of 2 hours. The plasma luteinizing hormone and follicle-stimulating hormone levels of women participating in the two regimens of pergolide showed a temporary and not significant inhibition of 30% and 20%, respectively (data not shown). Serum prolactin results of patients receiving long-term treatment with pergolide were combined in a single group ( $n = 13$ ) and were compared to those obtained in women receiving bromocriptine ( $n = 9$ ). A similar inhibition of prolactin release was demonstrated with both agonists (Fig. 4). Maximum suppression occurred after 8 weeks of treatment with bromocriptine ( $153 \pm 47$  to  $18.6$  ng/ml) and 4 weeks with pergolide ( $232 \pm 85$  to  $19.9 \pm 9$  ng/ml), at which time plasma levels reached a plateau that lasted until the completion of the study. To obtain these levels of prolactin inhibition, five patients required 7.5 mg of bromocriptine daily and four required 5 mg. Of those treated with pergolide, one received 300 µg, 10 received 100 µg, and two received 50 µg daily. Surprisingly, the patterns of serum luteinizing hormone and follicle-stimulating hormone secretion were different between the two dopamine agonists (Fig. 5). Starting at the fourth week of treatment patients receiving pergolide demonstrated significantly greater values ( $p < 0.05$ ) of both plasma luteinizing hormone and follicle-stimulating hormone levels than patients treated with bromocriptine. The inhibition of serum prolactin concentration with pergolide and bromocriptine was similar in women who bled and those who did not bleed following the administration of progesterone. There was no significant difference in the length of treatment of each agonist necessary to induce spontaneous menses and to correct the galactorrhea (Table II). The two patients with macroadenomas who were treated with pergolide had a significant reduction of the adenoma size as demonstrated by a computed tomographic scan repeated after 6 months of treatment. The size of the microadenoma of the other five patients treated with either drug did not change. Minor temporary side effects were reported by five patients treated with bromocriptine and 11 receiving pergolide; however, the difference was not significant. These side effects consisted of nausea, constipation, and dizziness and lasted for a few days at the

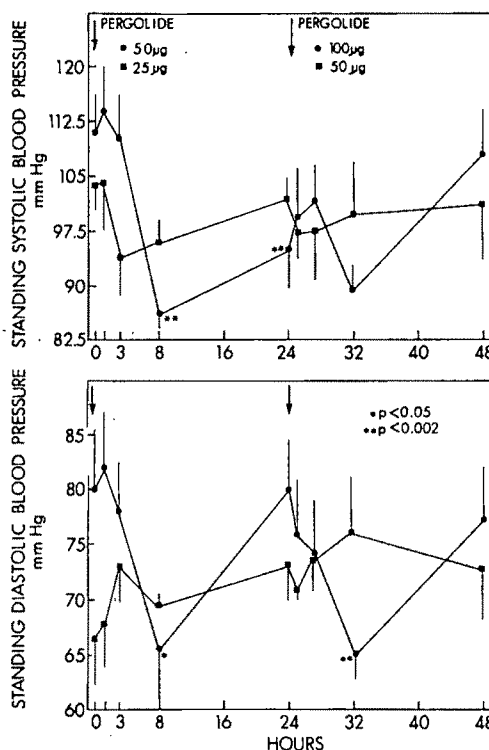


Fig. 1. Acute effect of pergolide on standing systolic and diastolic blood pressure. Nine women received 50 µg followed by 100 µg daily and three women received 25 µg followed by 50 µg.

Table II. Weeks of treatment to resolve symptoms (mean ± SEM)

Symptoms	Bromocriptine (n = 9)	Pergolide (n = 11)
Amenorrhea	6.9 ± 1.2	10.0 ± 2.4
Galactorrhea	7.5 ± 1.4	8.3 ± 1.8
Infertility	10 ± 1.1 (n = 4)	45 (n = 1)

beginning of treatment. In only two patients the side effects induced by 50 µg of pergolide were significant enough to discontinue treatment after 2 weeks.

### Comment

The results of this study revealed that both bromocriptine and pergolide mesylate are potent dopamine agonists that can be safely prescribed to a majority of patients with hyperprolactinemia. It was also demonstrated that the immediate side effects on blood pressure are dose related and can be diminished or avoided by initiating the treatment with a lower dose. As little as 25 µg daily of pergolide was sufficient to induce similar inhibitory effects on plasma prolactin, without significantly lowering blood pressure. Furthermore, the doubling of the dose to 50 µg in the second day of treatment induced an additional significant 60% suppression of baseline prolactin without any signifi-



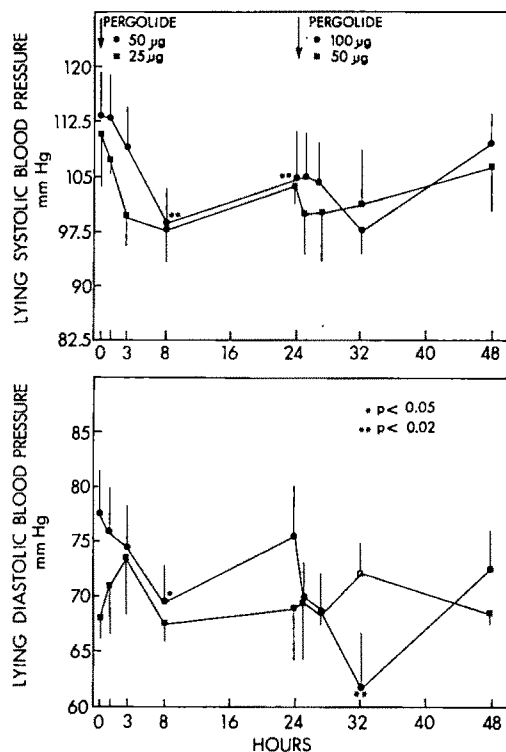


Fig. 2. Acute effect of pergolide on supine systolic and diastolic blood pressure in same patients as in Fig. 1.

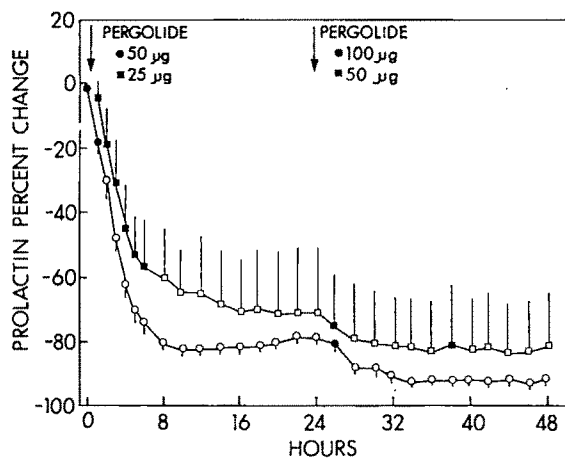


Fig. 3. Acute effect of pergolide on plasma prolactin with significant points represented by open symbols. Statistical comparisons were made between each point in time against the initial and the 24-hour baseline values (same patients as in Fig. 1).

cant increase in side effects or hemodynamic changes. In contrast, the initial administration of 50 µg followed by 100 µg was accompanied by significant alterations in blood pressure. Thus it can be concluded that treatment with pergolide should be initiated at 25 µg daily and increased only if necessary. Although not investigated in this study, bromocriptine has been reported

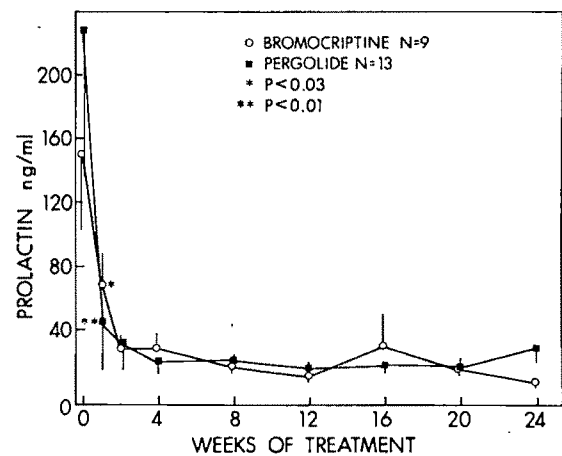


Fig. 4. Effect of long-term treatment with bromocriptine and pergolide on plasma prolactin. From week 4, 11 patients remained on pergolide (side effects). From week 12, there were seven patients, and from week 16, there were five patients receiving bromocriptine (pregnancy).

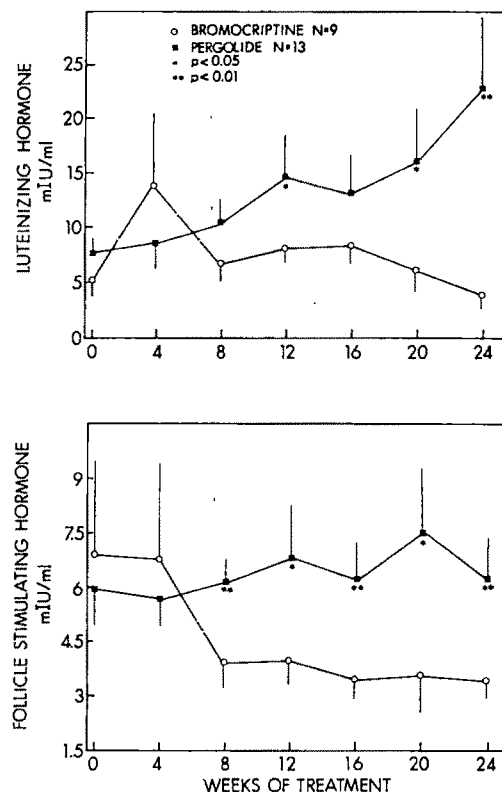


Fig. 5. Effect of long-term treatment with bromocriptine and pergolide on plasma luteinizing hormone and follicle-stimulating hormone in the same patients as in Fig. 4.

to lower the blood pressure of patients with essential hypertension and normotensive individuals.<sup>11, 12</sup> This circulatory dose-dependent effect of dopamine agonists has also been reported to occur when increasing doses of dopamine are given. Low doses of dopamine

either do not change or slightly reduce blood pressure by acting on dopamine receptors, and higher doses increase blood pressure by stimulating  $\alpha$ -adrenergic receptors.<sup>13, 14</sup> The administration of dopamine agonists induces hypotension by reducing central dopamine turnover and also by competing for the peripheral receptors on noradrenergic neurons resulting in diminished norepinephrine secretion.<sup>15</sup> A similar dynamic in the binding to all dopamine receptors can be assumed, since the maximum effect on blood pressure and prolactin secretion occurred 8 hours following pergolide administration. As previously reported, dopamine agonists inhibit prolactin release primarily by a selective binding on postsynaptic dopamine receptors present in the lactotrophs.<sup>16</sup> The finding that a pergolide/bromocriptine ratio of 25  $\mu$ g/2.5 mg in a single daily dose is sufficient to maintain prolactin levels within normal range supports the concept of a greater affinity of pergolide to dopamine receptors.<sup>17</sup> However, from a clinical point of view both agonists should be considered to be equipotent. In fact, this study demonstrates that in both groups of patients a similar pharmacologic effect in lowering serum prolactin concentration, cessation of galactorrhea, and resumption of ovulatory menstrual cycles was obtained in agreement with other reports.<sup>4, 5</sup> The finding of significantly elevated levels of both luteinizing hormone and follicle-stimulating hormone in women receiving pergolide for several weeks is intriguing. This difference was not due to either an increased number of ovulations in the group or to earlier resumption of ovulatory cycles. Clemens (personal communication) demonstrated that chronic administration of 1 and 3 mg/kg of pergolide to male rats induced a significant increase in serum luteinizing hormone. Further studies are necessary to elucidate the meaning of such unexpected difference, since initially pergolide treatment induced a temporary but not significant suppression of both luteinizing hormone and follicle-stimulating hormone secretion. In contrast, Perryman et al.<sup>18</sup> have reported a significant inhibition of plasma luteinizing hormone in normal men receiving 100  $\mu$ g of pergolide. In agreement with other reports we have also observed the shrinking effect of pergolide on the macroadenomas of two patients.<sup>5</sup> However, such effect was not evident in patients harboring a microadenoma.

In conclusion, either dopamine agonist can be safely administered to patients with hyperprolactinemia with or without a prolactin-secreting pituitary adenoma. A similar inhibitory effect on prolactin release and amelioration of clinical symptoms and signs should be expected.

We wish to express our gratitude to the National Pituitary Agency, Bethesda, Maryland, for their gen-

erosity in providing the reagents for the radioimmunoassay of prolactin, luteinizing hormone, follicle-stimulating hormone, and growth hormone.

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# Effect of aminophylline on lung maturation in preterm rabbit fetuses

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Pregnant rabbit does were treated intravenously with aminophylline (6 mg/kg/day) from the twenty-fifth day after the day of mating, and the fetuses were delivered by hysterotomy on the twenty-eighth day. One group of neonates was breathing air, and another group 100% oxygen. Lung mechanics were evaluated in the newborn animals during spontaneous or artificial ventilation, and the lungs were studied histologically with particular reference to the alveolar volume density. In one series of experiments, the lungs were washed and the lavage fluid was analyzed for phosphatidylcholine and phosphatidylglycerol.

Aminophylline-treated litters had greater body weights, an improved survival rate, and an increased amount of phosphatidylglycerol in lung lavage fluid. Respiratory frequency was increased in aminophylline-treated animals breathing air, but data on lung compliance showed no significant difference between treated and control animals. In the present model, the beneficial effect of aminophylline can be attributed largely to a combination of accelerated fetal growth and improved postnatal regulation of breathing and less to a specific influence on the biochemical and functional maturation of the lung. (AM J OBSTET GYNECOL 1986;154:436-9.)

**Key words:** Aminophylline, fetal lung maturation, phosphatidylcholine, phosphatidylglycerol

Data from animal experiments<sup>1,2</sup> indicate that lung adaptation can be facilitated in the premature neonate by maternal treatment with the phosphodiesterase inhibitor aminophylline and that the mechanisms by which this effect is obtained are in part similar to those operating after treatment with  $\beta$ -adrenergic agents or glucocorticoids. These mechanisms include an increase of cyclic adenosine monophosphate in fetal lung tissue,<sup>3</sup> which may promote glycogenolysis,<sup>4</sup> increase the content of tissue phospholipids,<sup>5</sup> and stimulate the production of surfactant phospholipids by the alveolar type II cells.<sup>6-9</sup> Direct injection of aminophylline into the premature fetus may, according to some authors,<sup>10</sup> induce a release of surfactant phospholipids into the alveolar spaces, although other investigators<sup>11</sup> have failed to confirm this effect.

In view of the possibility that aminophylline might be used in the prophylaxis of the neonatal respiratory

distress syndrome as an alternative to glucocorticoids,<sup>12</sup> we wanted to analyze whether maternal administration of this drug would improve in vivo lung mechanics and survival in prematurely delivered newborn rabbits. Since aminophylline may have a stimulatory effect on the central nervous system, including the units involved in the regulation of breathing,<sup>13</sup> we were interested in to what extent the effect would depend on whether the animals were breathing air or 100% oxygen. We also evaluated the effect of antenatal aminophylline treatment on the phospholipid profile of material sampled from the fetal air spaces by lung lavage.

## Material and methods

The experiments were carried out on a total of 163 premature newborn rabbits, obtained from 19 New Zealand white does on the twenty-eighth day after the day of mating (term = the thirty-first day). Aminophylline was administered intravenously from the twenty-fifth to the twenty-seventh day in two daily injections, 6 mg/kg/day. Control rabbits received the same number of injections with normal saline solution.

On the twenty-eighth day, the pregnant rabbits were killed by a rapid injection of 2 ml of pentobarbital sodium (40 mg/ml) and 5 ml of potassium chloride (150 mg/ml). The fetuses were immediately obtained by hysterotomy. After delivery, the neonates were weighed and tracheotomy was performed. In one series of experiments (protocol 1), the animals were allowed to breathe air spontaneously for 2 hours; in another series (protocol 2), the animals breathed 100% oxygen for 1

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**Table I.** Body weight, survival rate, lung mechanics, and alveolar volume density in premature newborn rabbits after maternal treatment with aminophylline and in controls (protocol 1)

Parameters	Group		p
	Aminophylline (n = 33)	Controls (n = 35)	
Body weight (gm) (mean $\pm$ SEM)	35 $\pm$ 1	31 $\pm$ 1	<0.005
Survival rate (%)			
30 min	67	46	NS
60 min	33	9	<0.05
120 min	30	9	<0.05
Respiratory frequency (breaths per min) (mean $\pm$ SEM)			
30 min	40 $\pm$ 10	16 $\pm$ 6	=0.05
60 min	56 $\pm$ 13	69 $\pm$ 3	NS
120 min	99 $\pm$ 12	70 $\pm$ 14	NS
C <sub>dyn</sub> (ml/cm H <sub>2</sub> O/kg) (mean $\pm$ SEM)			
30 min	0.56 $\pm$ 0.11	0.43 $\pm$ 0.15	NS
60 min	0.96 $\pm$ 0.16	0.62 (n = 1)	—
120 min	0.81 $\pm$ 0.38	*	—
V <sub>v</sub> (mean $\pm$ SEM)	0.41 $\pm$ 0.03	0.39 $\pm$ 0.03	NS

C<sub>dyn</sub> = Dynamic lung compliance; V<sub>v</sub> = alveolar volume density.

\*No successful recordings.

hour and were then subjected to artificial ventilation for 1 hour.

**Protocol 1.** The animals were kept in an incubator at 37° C. Lung mechanics during spontaneous ventilation were recorded 30, 60, and 120 minutes after birth, as described by Lachmann et al.<sup>14</sup> For this purpose, we used a fluid-filled esophageal catheter, a Fleisch tube connected to the tracheal cannula, a differential pressure transducer (EMT 32, Siemens-Elerna, Solna, Sweden), an integrator unit (EMT 41, Siemens-Elerna), and a recorder (Physiograph 6C, Narco Medical Systems, Dallas, Texas). Between the recordings the neonates were stimulated intermittently by gentle handling. Animals still making respiratory efforts 120 minutes after delivery were counted as survivors.

At the end of the experiment, all animals received an overdose of intraperitoneal sodium pentobarbital. The lungs were fixed in situ by immersion of the unopened thorax in formalin. Paraffin sections from the lower lobes were studied histologically with particular reference to the expansion pattern. The volume density of the alveolar compartment was determined by point-counting, using the total parenchyma as reference volume.

**Protocol 2.** After tracheotomy the neonates were kept at 37° C in a system of multiple individual body plethysmographs, flushed with 100% oxygen. Respiratory frequency was evaluated 30 and 60 minutes after birth, by connecting the tracheal cannula to the Fleisch tube system described above.

After the 60-minute recording, the animals were paralyzed with intraperitoneal pancuronium bromide (0.2 mg/ml, 0.1 ml) and connected in parallel to a pressure-constant ventilator system delivering 100% oxygen at a frequency of 40/min, and an inspiration:expiration

ratio of 1:1. Tidal volumes were recorded pneumotachographically, by connecting the Fleisch tube to the body plethysmograph. The insufflation pressure was adjusted to maintain an average tidal volume of about 10 ml/kg in each litter. In order to assess survival, recordings of electrocardiogram were obtained at the end of the experiment. The animals were then put to death with an overdose of intraperitoneal pentobarbital sodium.

In 42 animals (21 aminophylline-treated, 21 controls), the lungs were fixed by vascular perfusion. A cannula was inserted into the pulmonary trunk, and the lungs were expanded by raising the intratracheal pressure to 30 cm H<sub>2</sub>O. This pressure was then lowered to 10 cm H<sub>2</sub>O for 10 minutes, while the lungs were perfused via the pulmonary artery with a mixture of 1% glutaraldehyde and 3.5% formaldehyde at a pressure of 65 cm H<sub>2</sub>O. The lungs were stored in 10% formaldehyde for at least 24 hours and embedded in paraffin. Histologic sections from the lower lobes were examined morphometrically as described above.

In 39 animals (18 aminophylline-treated, 21 controls), the lungs were used for biochemical studies. These lungs were lavaged four times with 20 ml of saline per kilogram of body weight. Concentrations of phosphatidylcholine and phosphatidylglycerol were analyzed enzymatically according to Muneshige et al.<sup>15</sup>

**Statistical evaluation.** The  $\chi^2$  test and the Wilcoxon two-sample (two-tailed) test were used for statistical evaluation of our results.

## Results

**Protocol 1.** In litters treated with aminophylline, the average body weight was increased by approximately 13% and the survival rate was significantly improved 60 and 120 minutes after birth (Table I). The rate of



**Table II.** Body weight, survival rate, lung mechanics, and alveolar volume density in premature newborn rabbits after maternal treatment with aminophylline and in controls (protocol 2)

Parameter	Group		p
	Aminophylline (n = 43)	Controls (n = 52)	
Body weight (gm) (mean $\pm$ SEM)	40 $\pm$ 1	33 $\pm$ 1	<0.005
Survival rate (%)			
30 min	80	60	<0.05
60 min	73	60	NS
120 min	71	59	NS
Respiratory frequency (breaths per min) (mean $\pm$ SEM)			
30 min	101 $\pm$ 12	72 $\pm$ 12	NS
60 min	98 $\pm$ 9	88 $\pm$ 9	NS
C (ml/cm H <sub>2</sub> O/kg) (mean $\pm$ SEM)			
60 min	1.00 $\pm$ 0.09	0.76 $\pm$ 0.09	NS
120 min	0.85 $\pm$ 0.10	0.64 $\pm$ 0.09	NS
V <sub>v</sub> (mean $\pm$ SEM)	0.65 $\pm$ 0.12	0.62 $\pm$ 0.11	NS

C = Lung-thorax compliance; V<sub>v</sub> = alveolar volume density.

**Table III.** Phospholipids in lung lavage fluid (protocol 2)

Phospholipids	Group		p
	Aminophylline	Controls	
Phosphatidylcholine (nmol/ml)	15.2 $\pm$ 3.2	17.2 $\pm$ 3.8	NS
Phosphatidylglycerol (nmol/ml)	27.9 $\pm$ 6.8	9.7 $\pm$ 1.7	0.02

Values are given as mean  $\pm$  SEM.

respirations among survivors was higher in the treated animals 30 minutes after birth, but values for dynamic lung compliance and alveolar volume density showed no statistically significant differences when compared with those of the control group (Table I).

**Protocol 2.** Aminophylline-treated animals again had a higher body weight, and the survival rate was improved 30 minutes after birth (Table II). Respiratory frequency among survivors, lung compliance, alveolar volume density, and phosphatidylcholine in lung lavage fluid showed no statistically significant differences between treated animals and controls (Tables II and III). However, the concentration of phosphatidylglycerol in lavage fluid was increased in animals receiving aminophylline (Table III). There was no correlation between levels of phosphatidylglycerol in lung lavage fluid and respiratory frequency or between phosphatidylglycerol levels and lung-thorax compliance.

**Comparison and combination of data from protocol 1 and protocol 2.** Survival rate after 60 minutes was significantly higher in the neonates breathing 100% oxygen (protocol 2) than in those breathing air (protocol 1); this holds for both aminophylline-treated animals and controls ( $p < 0.001$ ). If survival figures from the two protocols are combined, there is again a statis-

tically significant improvement among the aminophylline-treated animals at the 30- and 60-minute intervals, compared with the combined control groups ( $p < 0.02$  and  $p < 0.05$ , respectively).

Aminophylline-treated neonates used for protocol 2 had a higher body weight than those used for protocol 1 ( $p < 0.005$ ); no such difference was found between the two control groups. The body weight remained significantly larger in the combined groups of aminophylline-treated animals than in the combined control groups, even if the individual litter was regarded as the sample: 38  $\pm$  1 gm ( $n = 9$ ) versus 34  $\pm$  2 gm ( $n = 10$ ) (mean  $\pm$  SEM;  $p = 0.05$ ).

Differences in alveolar volume density between animals subjected to protocol 1 and protocol 2 can be attributed entirely to the fact that in the former the lungs were immersed in formalin without distending pressure, whereas in the latter group an expanding pressure was maintained during perfusion fixation.

### Comment

Aminophylline exerts a wide range of pharmacologic actions, some of which are mediated by relaxation of the smooth muscles. For example, aminophylline inhibits uterine contractions and possibly increases uterine blood flow.<sup>15</sup> This is one of the reasons why aminophylline is used for the treatment of preterm labor and eventually for the treatment of fetal distress due to uterine hyperactivity.<sup>15, 16</sup> Another well-documented effect of aminophylline is bronchorelaxation, which makes it the traditional drug of choice for the treatment of asthma. Some of our results can be explained by the influence of aminophylline on the uterine tonus and the uteroplacental circulation. Enhanced uterine blood flow could lead to accelerated fetal growth, as indeed documented in the present series by increased average

fetal body weight in both groups of aminophylline-treated litters.

After maternal treatment with aminophylline, the fetuses were also more vigorous, with improved figures for survival and respiratory frequency. These may be nonspecific effects, reflecting the general maturity level in these particular litters. However, the increased respiratory frequency observed shortly after birth in aminophylline-treated animals breathing air (protocol 1) can also be explained by a direct action of the drug on the central nervous system. Aminophylline is widely used for the treatment of the apnea of prematurity because it increases the central "respiratory drive" of the newborn infant.<sup>17</sup>

Most previous studies on fetal rabbits have indicated that aminophylline accelerates the biochemical and functional maturation of the lung, as reflected by an increased production of surface-active phospholipids and improved pressure-volume characteristics.<sup>1, 2, 7-9</sup> In the present experiments, levels of phosphatidylglycerol were increased in lung lavage fluid from aminophylline-treated fetuses, suggesting accelerated lung maturity. We were unable to document a beneficial effect of aminophylline on the compliance of the lungs, either during spontaneous ventilation or during artificial ventilation. Average values for compliance were, in general, greater in aminophylline-treated animals than in controls, but there was a wide individual variation and the differences obtained with the present number of animals failed to reach statistical significance.

To a large extent, this variation may reflect the normal situation in rabbit fetuses on the twenty-eighth day. This day represents a transitional stage of fetal maturation, between the apneic, surfactant-deficient state on the twenty-seventh day and the nearly mature state on the twenty-ninth. Large standard errors are to be expected in parameters of lung maturation recorded in fetuses delivered on the twenty-eighth day. We nevertheless chose this gestational age for our study, as we wanted to record lung mechanics during spontaneous ventilation, also in control animals.

We conclude that the moderate beneficial effects of aminophylline, noted in the present study, can be attributed largely to a combination of accelerated fetal growth and improved postnatal regulation of breathing and only to a minor extent to a specific influence on biochemical and functional lung maturation.

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# Opioid regulation of pituitary gonadotropins and prolactin in women using oral contraceptives

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To determine the effect of oral contraceptives on endogenous opioid modulation of the hypothalamic-pituitary axis, we gave a bolus dose of 10 mg of naloxone intravenously in women using Lo/Ovral-28 oral contraceptives and in normal (control) women during the follicular (days 8 to 9) and luteal (days 21 to 23) phases. Plasma follicle-stimulating hormone, luteinizing hormone, and prolactin were measured by radioimmunoassay before and after naloxone at regular intervals. In oral contraceptive users ( $n = 5$ ) basal plasma follicle-stimulating hormone ( $3.7 \pm 0.4$  mIU/ml) and luteinizing hormone ( $3.2 \pm 0.5$  mIU/ml) levels were significantly lower than in control subjects during both follicular ( $10.7 \pm 0.9$  and  $16.7 \pm 2.0$ ) and luteal ( $7.7 \pm 1.4$  and  $10.0 \pm 0.9$ , respectively) phases ( $p < 0.05$  to  $< 0.001$ ). In contrast the basal plasma prolactin level was significantly higher in oral contraceptive users ( $25.0 \pm 4.1$  ng/ml) than in control subjects during the follicular ( $11.8 \pm 1.2$ ) and luteal ( $11.0 \pm 0.8$ ) phases ( $p < 0.01$ ). In control subjects, follicle-stimulating hormone, luteinizing hormone, and prolactin levels did not change significantly after naloxone in the follicular phase, but naloxone elicited a significant synchronous release of luteinizing hormone and prolactin during the luteal phase. In contrast, oral contraceptive users showed increases in luteinizing hormone and prolactin after naloxone that were not significantly different from the basal plasma levels. (AM J OBSTET GYNECOL 1986;154:440-4.)

**Key words:** Opioid, pituitary gonadotropins, prolactin, oral contraceptives, naloxone

The endogenous opiates present in the hypothalamus and the pituitary may have an inhibitory role in the neuroendocrine regulation of anterior pituitary function through control of the activity of hypothalamic gonadotropin-releasing hormone neurons. Studies with the use of the opiate antagonist naloxone in normal cycling women indicate that the modulating effects of the opiate may be influenced by ovarian steroid hormones.<sup>1-4</sup> Blockade of the opiate receptors with naloxone induced significant increases in luteinizing hormone (LH)<sup>1-3</sup> during the late follicular and luteal phases of the menstrual cycle and prolactin (PRL)<sup>2,4</sup> only during the luteal phase of the menstrual cycle. This suggests that endogenous opiates have a significant inhibitory effect on pituitary gonadotropin and PRL secretion during the luteal phase under the influence of both estrogen and progesterone.

Although the estrogen-progestogen contraceptive

pill is known to suppress the pituitary, there is little information on the effect of oral contraceptives on hypothalamic opioid activity. A preliminary study suggests that hypothalamic opioid activity may be elevated in women using oral contraceptives.<sup>5</sup> To elucidate the influence of oral contraceptives on the endogenous opiate system, we compared the effects of administering the opiate antagonist naloxone intravenously on days 18 to 20 in women using a low-dose oral contraceptive with the effects seen during the midproliferative and midluteal phases of the normal menstrual cycle.

## Material and methods

**Patients.** Five women, 19 to 26 years of age, who had been using the oral contraceptive Lo/Ovral-28 (0.3 mg of norgestrel and 0.03 mg of ethinyl estradiol) for 3 months for contraception were recruited for the study. The study was carried out in the fourth cycle of Lo/Ovral-28. The mean menstrual cycle length during use of the oral contraceptive was  $27.4 \pm 0.2$  ( $\pm$ SE) days, mean body weight was  $60.7 \pm 5.4$  kg, and blood pressure was 100 to 110/70 to 80 mm Hg. The controls were six normal women, 23 to 38 years of age, who had regular ovulatory menstrual cycles, as documented by biphasic basal body temperature patterns and high serum progesterone levels in the second half of the cycle. The mean menstrual cycle length was  $29.5 \pm 0.9$  days and mean body weight was  $61.2 \pm 4.9$  kg. The study was approved by the Institutional Review Committee

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**Table I.** Baseline hormone profile (mean  $\pm$  SE) during midfollicular and midluteal phases of women with normal cycles and women using oral contraceptives.

Hormone	Normal control subjects		c. Women using oral contraceptives (days 18-20)	Significance		
	a. Midfollicular phase	b. Midluteal phase		a vs. b	a vs. c	b vs. c
FSH (mIU/ml)	10.7 $\pm$ 0.9	7.7 $\pm$ 1.4	3.7 $\pm$ 0.4		p < 0.0001	p < 0.05
LH (mIU/ml)	16.7 $\pm$ 2.0	10.0 $\pm$ 1.0	3.2 $\pm$ 0.5	p < 0.001	p < 0.001	p < 0.001
Prolactin (ng/ml)	11.8 $\pm$ 1.2	11.0 $\pm$ 0.8	25.0 $\pm$ 4.1		p < 0.01	p < 0.01
Estrone (pg/ml)	135.7 $\pm$ 7.9	178.3 $\pm$ 14.2	79.1 $\pm$ 5.0	p < 0.025	p < 0.001	p < 0.001
Estradiol (pg/ml)	124.5 $\pm$ 8.9	179.6 $\pm$ 15.5	30.0 $\pm$ 2.3	p < 0.005	p < 0.001	p < 0.001
Progesterone (ng/ml)	1.8 $\pm$ 0.1	19.6 $\pm$ 1.0	1.8 $\pm$ 0.1	p < 0.001	p < 0.001	p < 0.001

The baseline hormone levels for each subject were obtained three times during 30 minutes before naloxone was given.

for Human Research and each woman gave written informed consent. For the control subjects, each was studied twice in the same cycle, once during the mid-follicular phase of the cycle (day 7 or 8) and once during the luteal phase (days 21 to 23). The oral contraceptive users were each studied once on days 18 to 20 of the cycle.

After an overnight fast, an indwelling needle with a heparin lock was inserted in a forearm vein. Thirty minutes later, three baseline heparinized blood samples were obtained at 15-minute intervals. A single bolus dose of 10 mg of naloxone was given intravenously during a 1-minute period after the third baseline blood sample was obtained. Heparinized blood samples were obtained 15, 30, 45, 60, 90, 120, and 180 minutes after naloxone injection. The blood was kept cold at 4° C and centrifuged. The plasma was removed and frozen at -20° C until assayed for follicle-stimulating hormone (FSH), LH, PRL, and baseline estradiol and progesterone levels.

**Hormone determinations.** To obviate interassay variability, all determinations for any single hormone were carried out on all of the plasma samples for all patients in the same assay.

Plasma FSH and LH were measured by a specific and sensitive double-antibody radioimmunoassay.<sup>6</sup> Details of these radioimmunoassays as performed in our laboratory have been given elsewhere.<sup>2</sup> For the LH radioimmunoassay, iodine 125-labeled LH was the radioactive ligand and the intra-assay coefficient of variation was 2.0% to 2.6%. For the FSH assay, <sup>125</sup>I-labeled FSH was the radioactive ligand and the intra-assay coefficient of variation was 3.3% to 7.7%. For both FSH and LH, the results were calculated as milli-international units of the second international reference preparation of human menopausal gonadotropin.

Plasma PRL was determined by a homologous double-antibody radioimmunoassay<sup>7</sup> with the use of a specific rabbit antihuman PRL antiserum with <0.001% cross-reactivity with FSH, LH, thyrotropin, human chorionic gonadotropin, and human growth hormone.

The intra-assay coefficient of variation was 3.3% to 6.8% and the PRL standard was prepared to correlate with the World Health Organization first international reference preparation 75/504.

Plasma unconjugated estradiol and estrone were measured by a specific and sensitive radioimmunoassay as previously described.<sup>8-10</sup> The limit of detection was 3 pg per assay tube and procedural losses were monitored by addition of tritiated estradiol and estrone. The final mean recovery for estradiol, including procedural losses, was 79.4%  $\pm$  1.1% ( $\pm$ SEM). All results were corrected for recovery.

Plasma progesterone was determined by a specific and sensitive radioimmunoassay<sup>11</sup> and the details as carried out in our laboratory have been described.<sup>9</sup> Procedural losses were monitored by the addition of tritiated progesterone. The final mean recovery, including extraction losses, was 73.2%  $\pm$  2.5% and all results were corrected for this.

To minimize variations between individuals in terms of circulating gonadotropins and PRL levels, their values were normalized to a mean basal level of 100%, which was the mean basal level calculated from the three prenaloxone basal hormone levels. All results after the administration of naloxone were calculated against this mean basal level of 100% for each of the hormones measured.

**Statistical analysis.** The mean ( $\pm$  SEM) plasma FSH, LH, and PRL levels after naloxone administration were compared with the mean of the three baseline levels with the use of both Student's nonpaired *t* test and analysis of variance, and the *p* value was obtained from a two-tailed table. All *p* values of  $\leq 0.05$  were considered significant.

## Results

Table I summarizes the mean  $\pm$  SE of baseline plasma FSH, LH, PRL, estrone, estradiol, and progesterone levels during the follicular and luteal phases of normal control subjects and in women using the oral contraceptive. Basal plasma FSH and LH levels were



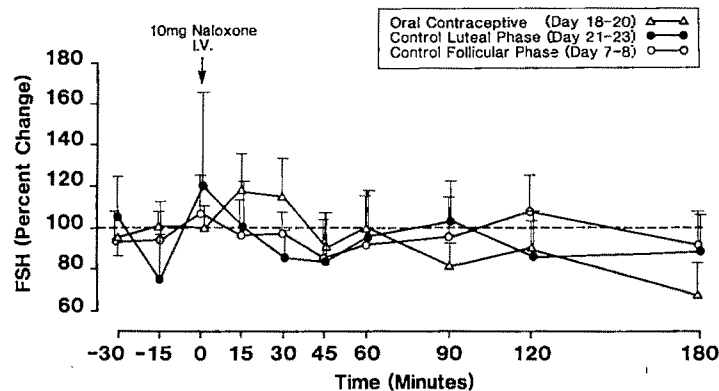


Fig. 1. Changes in plasma FSH levels from baseline (100%) after intravenous naloxone on days 18 to 20 in five women using oral contraceptive (Lo/Ovral-28) compared with those in normal control subjects during the follicular and luteal phases of the menstrual cycle. There was no significant change in FSH levels after intravenous (I.V.) naloxone in all subjects.

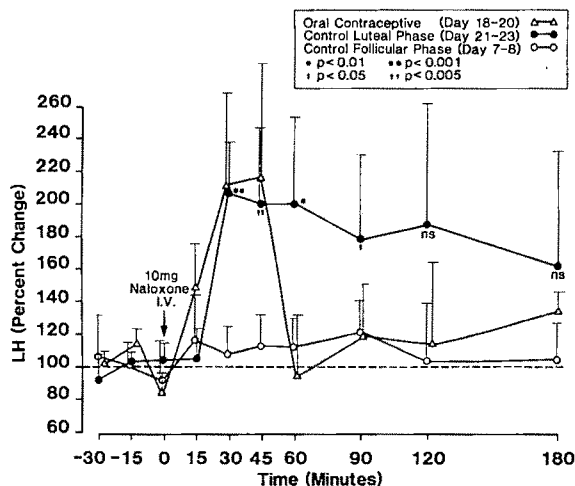


Fig. 2. Changes in plasma LH levels from baseline (100%) after intravenous naloxone on days 18 to 20 in five women using oral contraceptive (Lo/Ovral-28) compared with those in normal control subjects during the follicular and luteal phases of the menstrual cycle. There was a significant increase in LH levels at 30, 45, 60, and 90 minutes after naloxone compared with basal levels during the luteal phase in normal control subjects. Although LH increased at 30 and 45 minutes after naloxone, it was not significantly different from the basal levels in women using oral contraceptives. LH levels (in milliunits per milliliter) in women using oral contraceptives were always lower than the basal levels in the control women. I.V. = Intravenous; ns = not significant.

significantly lower in women using the oral contraceptive than during either the follicular or the luteal phase of normal control subjects ( $p < 0.05$  for each). The baseline plasma LH level was also significantly lower in the luteal phase than in the follicular phase in normal control subjects ( $p < 0.01$ ). In contrast, the baseline plasma PRL level was significantly higher than during either the follicular or the luteal phase in control subjects ( $p < 0.01$ ). Plasma estrone and estradiol levels

were lower in oral contraceptive users than in normal control subjects. Plasma progesterone levels were significantly lower than in the luteal phase and similar to those in the follicular phase of normal control subjects.

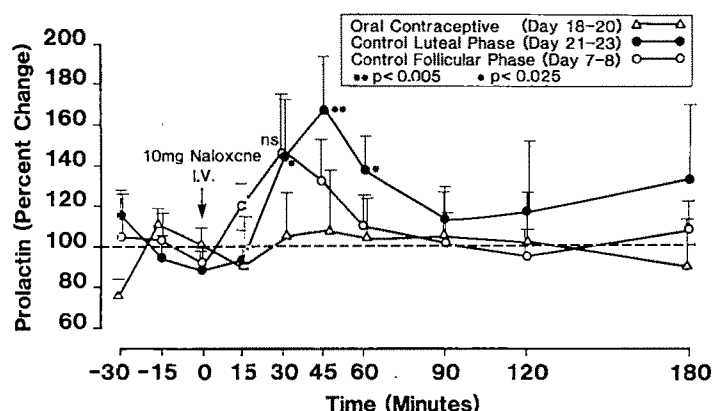
Plasma FSH changes from the mean baseline level (100%) in women using oral contraceptives and in normal control women during the follicular and luteal phases after 10 mg of naloxone given intravenously are shown in Fig. 1. There was no significant difference in plasma FSH changes in both normal control subjects during the follicular and luteal phases and women using oral contraceptives.

In normal control subjects, the plasma LH level did not change significantly after naloxone administration during the follicular phase. During the luteal phase, the plasma LH level increased significantly at 30, 45, 60, and 90 minutes after naloxone (Fig. 2). In women using oral contraceptives, there was a large but not significant increase in LH from the mean baseline levels after naloxone at 30 and 45 minutes.

Fig. 3 shows the changes in plasma PRL from the baseline levels (100%) after intravenous naloxone in control subjects and in women using oral contraceptives. While basal PRL levels were significantly higher in women using oral contraceptives (Table I), there was little change in the plasma PRL level after naloxone (Fig. 3). There was a significant rise in plasma PRL from basal levels at 30, 45, and 60 minutes after naloxone was given to control subjects during the luteal phase.

### Comment

In normal women, several studies,<sup>1,3</sup> including our previous observation,<sup>2</sup> have shown that there is a significant inhibitory effect of endogenous opioids on the release of pituitary LH during the luteal phase under the influence of high progesterone levels. Medroxyprogesterone acetate (Provera) also increases endoge-



**Fig. 3.** Changes in plasma PRL levels from baseline (100%) after intravenous naloxone on days 18 to 20 in five women using oral contraceptives (Lo/Ovral-28) compared with those in normal control subjects during the follicular and luteal phases of the menstrual cycle. There was a significant increase in PRL levels at 30, 45, and 60 minutes after naloxone compared with basal levels in the luteal phase of control subjects but not in women using oral contraceptives. I.V. = Intravenous; ns = not significant.

nous opioid peptide activity in postmenopausal women but the dose of 20 mg of Provera daily for 30 days is unusually high.<sup>12</sup> Under the influence of the low-dose oral contraceptive Lo/Ovral-28, which contains 0.03 mg of ethinyl estradiol and 0.3 mg of norgestrel, there is a large but not significant increase in the plasma LH level from baseline levels after administration of the opiate antagonist naloxone; the maximum levels of LH reached ( $6.7 \pm 2.2$  mIU/ml) were, however, still lower than baseline levels in normal control subjects (Table I). This suggests that the low-dose oral contraceptive has little or no significant increase in the endogenous opioid inhibitory activity when compared with the effects seen during the follicular phase of the menstrual cycle. The low levels of LH seen in women using the oral contraceptive may be due to a direct effect of the oral contraceptive on the gonadotropes, which is a reduction in the synthesis and pool of FSH and LH. Although the oral contraceptive Lo/Ovral-28 contains a very potent progestogen (norgestrel), the circulating blood levels of progesterone attained are similar to those of the mid-follicular phase. While the comparative biologic potency of progestogens is derived from their effect on the uterine tissue, their relative potencies on other target tissues such as the hypothalamus and brain may differ from that of the uterus. Therefore, oral contraceptives appear to have an inhibitory effect on pituitary gonadotropins through both a direct effect on the gonadotropes and an increase in hypothalamic opioid activity.

We have previously reported the synchronous release of prolactin and LH during the luteal phase of the normal cycle and postulated an inhibitory effect of endogenous opioids on the release of these two adenohypophyseal hormones.<sup>2</sup> This observation on prolactin

was recently confirmed,<sup>4</sup> thus refuting earlier observations of a direct prolactin stimulatory effect of endogenous opioids. Although the basal serum prolactin levels were increased with the use of oral contraceptives but still within the upper ranges of normal, we were unable to elicit any significant release of prolactin after opioid blockade with naloxone. The lack of a naloxone-induced PRL release with the low-dose oral contraceptive Lo/Ovral-28 could be due to either (1) the dose of estrogen-progestogen and the type of progestogen and its effects on the hypothalamus and/or (2) the low levels of circulating progesterone compared with the luteal phase progesterone levels of the normal cycle (Table I). The latter would further confirm earlier observations that under the influence of luteal phase progesterone levels, there is a maximal opioid inhibitory effect during the normal ovulatory cycle in women.

Our findings are at variance with a recent report of significant increases in prolactin levels during naloxone infusion in oral contraceptive users.<sup>5</sup> These differences may be partially explained by the 50  $\mu$ g or less of estrogen in the oral contraceptive used by the patients in that study whereas our patients were all using identical oral contraceptives containing only 30  $\mu$ g of ethinyl estradiol and 0.3 mg of norgestrel. Furthermore, norgestrel has potent androgenic effects and, unlike many of the 19-nortestosterone derivative, no estrogenic effect. Alternatively, we used an intravenous bolus dose of naloxone while continuous intravenous infusion for 4 hours was used in the earlier report.<sup>5</sup> Nevertheless, this is unlikely to account for the apparent differences as similar findings have been reported in normal menstrual cycles with both protocols of naloxone administration.<sup>2, 15</sup>

Elevation of endogenous opioid peptides and/or re-

ceptor activity in oral contraceptive users may partially account for some of the common side effects of oral contraceptive pills such as increased appetite, especially a craving for sweets, weight gain, depression, and libido changes. If the low-dose oral contraceptive containing 30 µg of ethinyl estradiol increased endogenous opioid activity less than the 50 µg dose, it could explain the lower prevalence (in the low-dose pill) of some of the side effects partially caused by the opioid activity. None of our study patients using Lo/Ovral-28 had any of these side effects, which may be explained by their modest increase in endogenous opioid activity.

Our observations on the effects of naloxone in women using the low-dose oral contraceptive compared to effects in normal cycling women further confirm the role and importance of estrogen and progesterone feedback on endogenous hypothalamic opioid peptide activity. In rats the estradiol-induced decrease in the  $\beta$ -endorphin content of the hypothalamus, thalamus, and midbrain can be blocked by progesterone,<sup>12</sup> while in the monkey hypophyseal portal blood levels of  $\beta$ -endorphin are increased after estrogen and progesterone treatment in ovariectomized animals.<sup>13</sup> In our patients using the low-dose oral contraceptive, plasma levels of estradiol and estrone were significantly lower than those in both the follicular and the luteal phases of the normal cycle but the plasma progesterone levels were similar to those in the midfollicular phase of the normal cycle. Thus the decreased estrogen:progesterone ratio may enhance endogenous opioid activity but not sufficiently high enough to be similar to that seen during the normal luteal phase.

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# Modulation of luteinizing hormone immunoreactivity and bioactivity by dopamine but not norepinephrine in women

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It has been suggested that changes in bioactive luteinizing hormone in women occur toward midcycle and may result in increased bioactive/immunoreactive luteinizing hormone ratios. To determine whether dopamine or norepinephrine modulate immunoreactive and bioactive luteinizing hormone secretion, 15 ovulatory women were studied in the mid to late follicular phase. Dopamine in two doses (0.5  $\mu\text{g/kg/min}$  and 4  $\mu\text{g/kg/min}$ ) and norepinephrine, 0.1  $\mu\text{g/kg/min}$ , were infused for 4 hours, and metoclopramide, 10 mg intravenously, was also given to determine whether dopamine receptor antagonism results in changes. Bioactive luteinizing hormone and the bioactive/immunoreactive luteinizing hormone ratio increased in women from the early to late follicular phase ( $p < 0.05$ ). Both intravenous doses of dopamine resulted in significant decrements in immunoreactive luteinizing hormone ( $20 \pm 2$  and  $20 \pm 3\%$ ,  $p < 0.02$ ) and bioactive luteinizing hormone (36.7% and 43.2%,  $p < 0.05$ ). With dopamine there was also a significant decrease in the bioactive/immunoreactive luteinizing hormone ratio ( $p < 0.02$ ). Intravenous norepinephrine, however, resulted in no changes in either immunoreactive or bioactive luteinizing hormone levels. Metoclopramide also did not change immunoreactive or bioactive luteinizing hormone levels. These data suggest that although endogenous dopaminergic blockade may not play a significant role in determining basal levels of luteinizing hormone, decreases in dopamine at the pituitary level may increase the bioactive/immunoreactive luteinizing hormone ratio. Norepinephrine does not appear to exert major changes in immunoreactive or bioactive luteinizing hormone. (AM J OBSTET GYNECOL 1986;154:445-50.)

**Key words:** Dopamine, norepinephrine, luteinizing hormone bioactivity

Recent investigation into the secretory dynamics of luteinizing hormone has confirmed its molecular heterogeneity in blood and indicated that standard assays for serum immunoreactive luteinizing hormone may not reflect its biological activity during certain conditions. Thus in menopausal patients, in polycystic ovary syndrome, and during most pulses of immunoreactive luteinizing hormone, including the luteinizing hormone surge, an increase in the ratio of bioactive/immunoreactive luteinizing hormone occurs.<sup>1-4</sup> These data favor the concept that conditions in which luteinizing hormone secretion is heightened result in greater bioactive luteinizing hormone secretion. However, gonadotropin-releasing hormone administration does not consistently increase the bioactive/immunoreactive luteinizing hormone ratio<sup>5</sup> although down regulation of the gonadotrope by a gonadotropin-releasing hormone agonist lowers this ratio.<sup>6</sup>

During the follicular phase of the menstrual cycle a gradual increase in the bioactive/immunoreactive luteinizing hormone ratio has been observed in women<sup>1</sup> and in the monkey.<sup>7</sup> Although this important increase in luteinizing hormone bioactivity toward midcycle may be explained by estrogen, under certain conditions estrogen may have an inhibitory effect on bioactive luteinizing hormone.<sup>2-8</sup> Two other putative neuromodulators of gonadotropin-releasing hormone and luteinizing hormone at midcycle are dopamine and norepinephrine. We intended, therefore, to determine the effects of dopamine and norepinephrine on the biologic activity of luteinizing hormone in women.

## Material and methods

**Subjects.** Fifteen normal, ovulating women, ages 20 to 37, were studied during days 7 to 12 of the menstrual cycle and received infusions of dopamine or norepinephrine or both. In addition, blood samples were obtained from four women as soon as dominant follicle diameters were 18 mm by ultrasonographic measurement. This was done to determine bioactive/immunoreactive luteinizing hormone ratios just prior to the spontaneous luteinizing hormone surge.

**Protocol.** Dopamine was infused for 4 hours at a rate of 0.5  $\mu\text{g/kg/min}$  (low dose) in five women and at a rate

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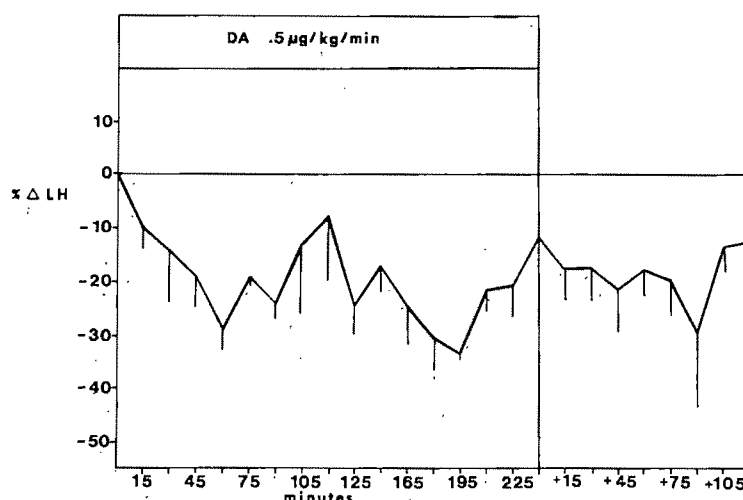


Fig. 1. Mean ( $\pm$ SEM) percent changes from basal levels of serum immunoreactive luteinizing hormone (LH) during dopamine (DA) infusion of  $0.5 \mu\text{g/kg/min}$  ( $p < 0.001$  compared to baseline values).

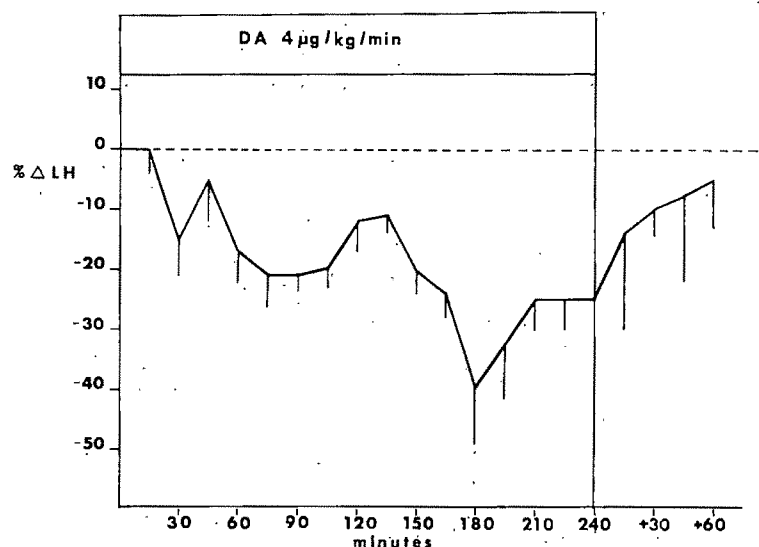


Fig. 2. Mean ( $\pm$ SEM) percent changes from basal levels of serum immunoreactive luteinizing hormone during dopamine infusion of  $4.0 \mu\text{g/kg/min}$  ( $p < 0.02$  compared to baseline values).

of  $4 \mu\text{g/kg/min}$  (high dose) in four women. The five women given the low-dose dopamine infusion also received a bolus of metoclopramide, 10 mg intravenously, 3 hours after the end of the dopamine infusion.

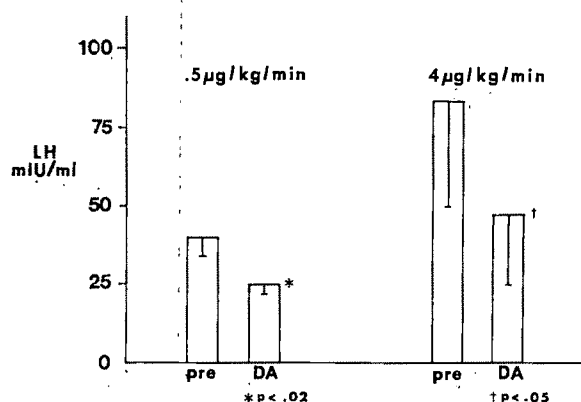
In six women, norepinephrine was infused at  $0.1 \mu\text{g/kg/min}$  for a period of 4 hours. Blood pressure and pulse rates were monitored at frequent intervals during each infusion.

Venous blood samples were drawn through an indwelling heparinized catheter every 15 minutes for 1 hour before, during, and for 1 hour after each infusion. Similarly, blood samples were drawn at 15-minute intervals for 1 hour before and for 3 hours after the metoclopramide bolus.

In this study no subject was given infusions of saline solution alone. In our other infusion studies in normal

women in which frequent sampling for luteinizing hormone was carried out for 4 to 6 hours before the test substance was administered, no significant changes in luteinizing hormone occurred over time when compared to baseline values.<sup>9</sup> Furthermore, as significant changes in luteinizing hormone with dopamine infusion had already been reported with a similar protocol,<sup>10</sup> we felt this additional testing was unnecessary.

Blood samples were allowed to clot and serum was separated and stored at  $-20^\circ\text{C}$  until assayed. Luteinizing hormone measurements by radioimmunoassay were performed as described previously with use of LER 907 as standard.<sup>11</sup> Reagents were generously provided by the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases. An in vitro bioassay for luteinizing hormone was performed with use of a



**Fig. 3.** Mean ( $\pm$ SEM) preinfusion (*pre*) and infusion levels of bioactive luteinizing hormone for dopamine infusions of 0.5  $\mu$ g/kg/min and 4  $\mu$ g/kg/min ( $p < 0.02$ , preinfusion versus infusion at 0.5  $\mu$ g/kg/min level;  $p < 0.05$ , preinfusion versus infusion at 4  $\mu$ g/kg/min level).

modification of the mouse interstitial cell assay as described previously.<sup>1</sup> In this bioassay, LER 907 was also used as the standard. The appropriateness of the use of this pituitary standard and the validation for the assay have been reported previously.<sup>1</sup> All samples from an individual patient were run in the same immunoassay or bioassay to reduce interassay variation. Intraassay and interassay coefficients of variation did not exceed 6% and 15%, respectively, for the immunoassays and bioassays.

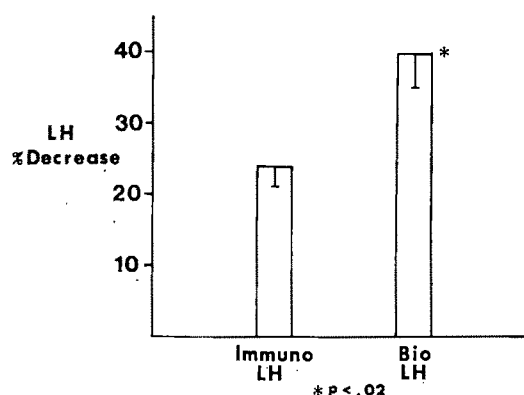
Luteinizing hormone values were log transformed, and statistical analyses were performed with use of two-tailed paired and unpaired Student's *t* test. A *p* value of  $<0.05$  was considered significant.

## Results

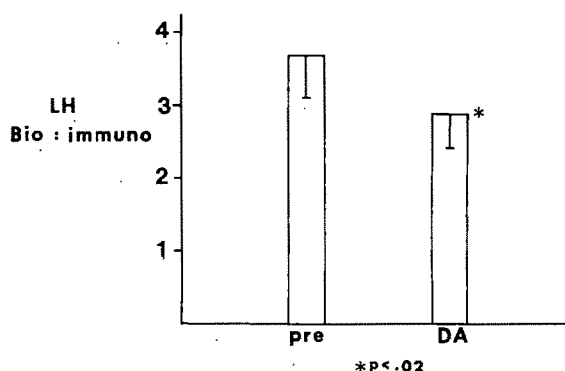
In four of the subjects studied, mean ( $\pm$ SE) bioactive luteinizing hormone increased from  $70 \pm 12$  mIU/ml on day 8 to  $284 \pm 75$  mIU/ml,  $p < 0.05$ , at follicle diameters of 18 mm, just before luteinizing hormone surge. The bioactive/immunoreactive luteinizing hormone ratio also increased from  $3.7 \pm 0.6$  to  $5.03 \pm 0.39$ ,  $p < 0.05$ .

In the hour of sampling, before dopamine or norepinephrine infusion, measurements of immunoreactive luteinizing hormone revealed fairly steady levels with only an 8% to 15% coefficient of variation in values. An example of this is the series of luteinizing hormone values of  $15.1 \pm 1$ ,  $15.1 \pm 1$ ,  $15.2 \pm 0.7$ ,  $17.4 \pm 1.6$ , and  $15.1 \pm 1.4$  mIU/ml which were obtained in the samples before obtaining 0.5  $\mu$ g/kg/min of dopamine. Changes in bioactive luteinizing hormone were also fairly constant with a coefficient of variation of 9% to 30% and values of  $76.1 \pm 24$ ,  $72.5 \pm 18.5$ , and  $68.4 \pm 22$  mIU/ml obtained before infusion.

There were no conclusive changes noted in any subject during dopamine infusion at low or high doses.



**Fig. 4.** Mean ( $\pm$ SEM) percent decrease in immunoreactive luteinizing hormone and bioactive luteinizing hormone levels during dopamine infusion ( $p < 0.02$ , immunoreactive luteinizing hormone versus bioactive luteinizing hormone).



**Fig. 5.** Mean ( $\pm$ SEM) bioactive/immunoreactive luteinizing hormone ratio before and during dopamine infusion ( $p < 0.02$ , before versus during).

Baseline bioactive luteinizing hormone and immunoreactive luteinizing hormone values were  $39.8 \pm 5.6$  and  $15.6 \pm 0.5$  mIU/ml, respectively, for the subjects on the day of the low-dose dopamine infusion. Baseline values for the subjects receiving high-dose dopamine infusion were similar ( $84.5 \pm 33.8$  and  $15.6 \pm 1.8$  mIU/ml). Although baseline bioactive/luteinizing hormone was higher in subjects receiving the higher dose, this was not a significant difference. Responses of immunoreactive luteinizing hormone to low- and high-dose dopamine infusions were similar (Figs. 1 and 2). Serum immunoreactive luteinizing hormone decreased significantly during dopamine infusion for both doses ( $p < 0.001$ , low dose;  $p < 0.02$ , high dose). The mean percent decrease in immunoreactive luteinizing hormone over the infusion period was similar for the low and high doses ( $20\% \pm 2\%$  and  $20\% \pm 3\%$ , respectively). The percent decrease was calculated from the change in luteinizing hormone between baseline values and those obtained after 120 minutes of dopamine infusion, when steady state conditions occurred.

Bioactive luteinizing hormone values were deter-

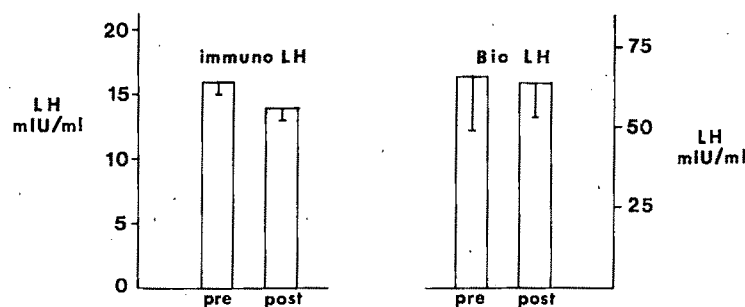


Fig. 6. Mean ( $\pm$ SEM) immunoreactive luteinizing hormone and bioactive luteinizing hormone levels before and after metoclopramide administration, 10 mg intravenously.

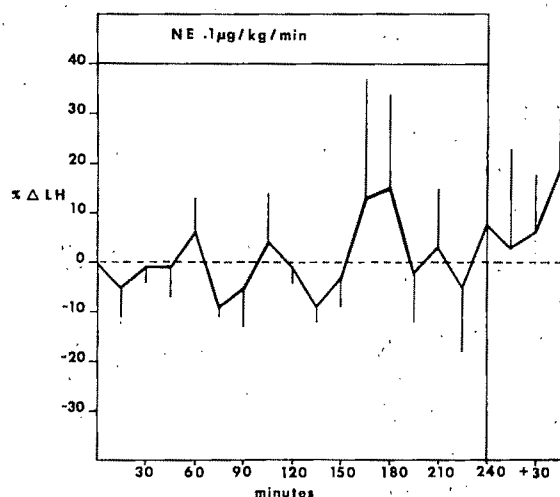


Fig. 7. Mean ( $\pm$ SEM) percent changes from basal levels of serum immunoreactive luteinizing hormone during norepinephrine (NE) infusion of 0.1  $\mu$ g/kg/min.

mined in all preinfusion samples and in at least four of the samples 120 minutes after dopamine infusion when steady state had been achieved. The decrement in luteinizing hormone values was sustained after 120 minutes and did not vary statistically. Calculations for the percent decrease in bioactive luteinizing hormone were carried out in the same way as for immunoreactive luteinizing hormone. Bioactive luteinizing hormone responses after dopamine infusion were similar for the two infusion doses. In the subjects receiving low-dose dopamine, bioactive luteinizing hormone decreased from  $39.8 \pm 5.6$  to  $24.9 \pm 2.7$  mIU/ml ( $p < 0.02$ ) for a mean percent decrease of 36.7% (Fig. 3). Bioactive luteinizing hormone in the high-dose recipients decreased from  $84.5 \pm 33.8$  mIU/ml to  $48.5 \pm 23.6$  mIU/ml ( $p < 0.05$ ) for a mean percent decrease of 43%  $\pm$  2% (Fig. 3).

When the grouped data of immunoreactive and bioactive luteinizing hormone responses to dopamine are compared (Fig. 4), bioactive luteinizing hormone exhibited a significantly greater decrement

(40%  $\pm$  5%) after dopamine compared to the mean percent decrease in immunoreactive luteinizing hormone (20%  $\pm$  3%,  $p < 0.02$ ). As a result the bioactive/immunoreactive luteinizing hormone ratio decreased from  $3.7 \pm 0.6$  to  $2.9 \pm 0.5$  ( $p < 0.02$ ) (Fig. 5).

Although exogenous dopamine significantly altered both immunoreactive luteinizing hormone and bioactive luteinizing hormone, blockade of endogenous dopamine by metoclopramide had no effect (Fig. 6). Baseline bioactive luteinizing hormone and immunoreactive luteinizing hormone were  $66.3 \pm 17.1$  and  $16.1 \pm 1.3$  mIU/ml, respectively. Following metoclopramide administration, no significant change in bioactive or immunoreactive luteinizing hormone level was observed at any time point over the 3-hour sampling period. Mean bioactive and immunoreactive luteinizing hormone after metoclopramide administration were  $64.1 \pm 11.5$  and  $14.1 \pm 1.1$  mIU/ml, respectively.

Norepinephrine infusion resulted in an increase in mean blood pressure with a maximum increase of  $34 \pm 6$  mm Hg occurring 24 minutes after initiation of norepinephrine. No significant changes in pulse were observed. Baseline immunoreactive luteinizing hormone value,  $19.2 \pm 4.6$  mIU/ml, was unaltered during norepinephrine infusion, with a mean infusion value of  $17.6 \pm 4.0$  mIU/ml (Fig. 7). Similarly baseline bioactive luteinizing hormone value,  $70.0 \pm 22.1$  mIU/ml, remained unchanged during norepinephrine with a mean infusion value of  $69.1 \pm 20.9$  mIU/ml.

### Comment

Dopamine infusion at doses of approximately 4  $\mu$ g/kg/min has consistently produced a decrease in immunoreactive luteinizing hormone similar to the one reported here.<sup>10, 12, 15</sup> However, to obtain serum levels of dopamine similar to levels observed in portal blood in primates, much lower doses of dopamine are required.<sup>14-17</sup> This more physiologic dose is equivalent to the dose of 0.5  $\mu$ g/kg/min infused in this study. In our subjects, increasing the dose from 0.5 to 4.0  $\mu$ g/kg/min did not alter the response of either bioactive or im-

munoreactive luteinizing hormone, suggesting that maximum responses of dopamine infusion may be achieved with 0.5  $\mu\text{g/kg/min}$ , confirming a recent report.<sup>15</sup>

We demonstrate here that there was a significantly greater decrement in bioactive luteinizing hormone compared to immunoreactive luteinizing hormone after dopamine infusion, resulting in a decreased bioactive/immunoreactive luteinizing hormone ratio. These data are compatible with a direct inhibitory role of dopamine in modulating the secretion of bioactive luteinizing hormone from the pituitary. Our findings, however, that metoclopramide was unable to alter either bioactive or immunoreactive luteinizing hormone by blocking endogenous dopamine activity places doubt on the physiologic significance of dopamine in modulating basal levels of luteinizing hormone.

Although in the early follicular phase the bioactive/immunoreactive luteinizing hormone ratio does not change when luteinizing hormone is stimulated by exogenous gonadotropin-releasing hormone, during a spontaneous luteinizing hormone pulse the bioactive/immunoreactive luteinizing hormone ratio does increase.<sup>1,4</sup> It is plausible, therefore, that during a spontaneous pulse or surge of luteinizing hormone that a concomitant decrease in dopaminergic tone, occurring only for a short time, may result in increased bioactive luteinizing hormone and elevated bioactive/immunoreactive luteinizing hormone ratios. During the luteinizing hormone surge, the change in dopamine value may in turn be mediated by changes in estradiol.

We are unaware of any previous reports on the effect of norepinephrine on luteinizing hormone levels in humans. In animal studies, norepinephrine has been shown primarily to stimulate luteinizing hormone secretion via both hypothalamic and pituitary  $\alpha$ -adrenergic receptors.<sup>16</sup> Norepinephrine infusion in our subjects caused only initial mild elevations of blood pressure, but there were no alterations noted in serum bioactive or immunoreactive luteinizing hormone values. This would imply that norepinephrine at this level of dose in women has little, if any, direct pituitary effect on either bioactive or immunoreactive luteinizing hormone secretion. Only if the effect of norepinephrine on luteinizing hormone was inhibitory, an effect opposite to that expected,<sup>16</sup> might the pressor effects of norepinephrine on clearance have negated our ability to demonstrate a modulatory role. However, it is still possible that norepinephrine may exert a modulating role on the gonadotropin-releasing hormone neuron at the level of the hypothalamus.

Although there are many factors involved in the regulation of luteinizing hormone secretion and in the differences in its bioactivity and immunoactivity, we have chosen to examine the effects of two neurotransmitters,

dopamine and norepinephrine. It appears that while dopamine does play a modulatory role on immunoreactive and bioactive luteinizing hormone and the immunoreactive/bioactive luteinizing hormone ratio, the effects of norepinephrine, if existent, are more elusive.

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## Failure to demonstrate decreased $\beta$ -adrenergic receptor concentration or decreased agonist efficacy in term or preterm human parturition

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Iodine 125-labeled iodocyanopindolol, a radioactive  $\beta$ -adrenergic antagonist, bound to particulate preparations of pregnant human myometrium in a manner compatible with binding to the  $\beta$ -adrenergic receptor. Studies with a specific  $\beta_2$ -antagonist, IPS 339, indicated that 72% of receptors present were of the  $\beta_2$ -subtype. Quantitative studies of  $\beta$ -receptor concentrations in myometrium from women at term indicated no change in receptor concentration during labor. Similarly, there was no difference in  $\beta$ -receptor concentration in myometrium from women in labor or before labor between 28 and 34 weeks of gestation. Concentrations of the  $\beta$ -receptor were not different at any stage of gestation assayed. Isoproterenol competition for iodocyanopindolol binding was used to examine efficacy of receptor agonist interactions in myometrium from women at term, in labor, or before labor and from women in preterm labor. There was no difference in high-affinity binding, an index of efficacy, in any of the groups examined. (*AM J OBSTET GYNECOL* 1986;154:450-6.)

**Key words:** Parturition, uterus,  $\beta$ -adrenergic receptor

The adrenergic response of the myometrium is influenced by exogenous gonadal steroids. In studies of rabbit uteri, adrenergically stimulated myometrial contractility, sensitivity, and concentration of  $\alpha$ -adrenergic receptors increased after pretreatment with estrogen. When estrogen treatment was followed by progesterone, the predominant adrenergic response was inhibition of contractility mediated by  $\beta$ -adrenoreceptor activation.<sup>1</sup>

In human studies, adrenergic myometrial response at different phases of the reproductive cycle mimicked the results from rabbit studies. Women given intravenous epinephrine during the follicular phase of the cycle (high estrogen) demonstrated increased uterine

**Table I.** Indications for cesarean section in term pregnancy

	Labor	No labor
Elective repeat	0	7
Breech	3	1
Cephalopelvic disproportion	6	0
Diabetes mellitus	0	2
Carcinoma, cervix	0	1
Chorioamnionitis	1	0
Total	10	11

contractility. In contrast, intravenous epinephrine administered to women during the luteal phase (high progesterone) failed to increase uterine contractility.<sup>2</sup> The ability to inhibit uterine contractions by activation of  $\beta$ -adrenergic receptor (myometrial relaxation) has widespread clinical application for the treatment of preterm labor.

The reported modulation of myometrial adrenergic receptor response by gonadal steroids and the potent effects of  $\beta$ -receptor activation to inhibit uterine contractions led us to hypothesize that endocrine changes preceding parturition might decrease  $\beta$ -adrenergic receptor concentration or efficacy of receptor agonist in-

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**Table II.** Indications for cesarean section in preterm pregnancy

Gestational age (wk)	Labor	Rupture of membranes	Medications	Delivery indication	$R_i$ (fmol/mg)	$K_d$ (pmol/L)
28	Yes	No	None	Breech	33	26
28	Yes	Yes	Ritodrine, betamethasone	Chorioamnionitis, breech	11	17
29	Yes	No	Magnesium sulfate	Intrauterine growth retardation, maternal cardiomyopathy, renal failure	23	20
29	Yes	Yes	Bethamethasone	Chorioamnionitis, breech	12	10
30	Yes	Yes	Ritodrine, betamethasone	Breech	40	19
30	No	No	Betamethasone	Maternal congestive heart failure	12	10
31	Yes	Yes	Ritodrine, betamethasone, magnesium sulfate	Maternal severe preeclampsia	8	10
31	No	Yes	Ritodrine, betamethasone	Breech	49	20
32	No	No	None	Maternal hypertension	38	21
34	No	No	None	Intrauterine growth retardation, maternal chronic renal failure	8	—
34	Yes	Yes	None	Diabetes	13	26

teractions and thereby be involved in the initiation of labor.

With use of radioligand iodine 125-labeled iodocyanopindolol we determined  $\beta$ -receptor concentrations in myometrium. We quantitated  $\beta$ -receptor concentrations in myometrium from women in labor and before labor, at term, and between 28 and 34 weeks' gestation. Our data do not support a role for decreased  $\beta$ -adrenoreceptors to initiate labor, since we found no change in the concentration of  $\beta$ -receptors or the efficacy of agonist receptor interactions in any of these groups.

### Material and methods

Myometrial strips were removed from the lower uterine segment of pregnant women at the time of cesarean section for obstetric indications (Tables I and II). All myometrial strips were obtained after receiving informed patient consent in accordance with the Human Rights Committee of the University of California (San Francisco). Term pregnant women received no medications before sampling, with the exception of anesthetic agents. Many of the women in preterm labor received ritodrine or terbutaline to inhibit uterine contractions. Some patients also received glucocorticoids to enhance fetal lung maturation (Table II).

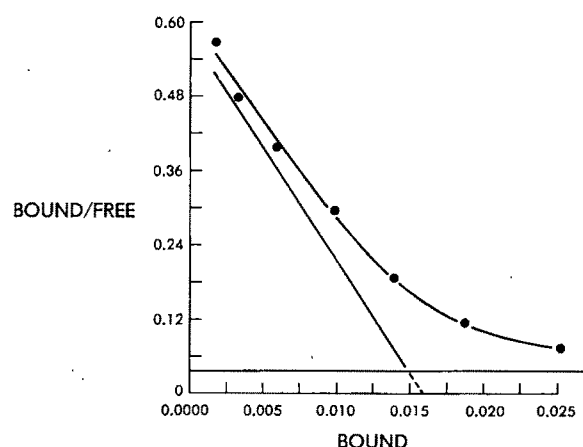
After delivery of the fetus and placenta, 2 × 6 cm myometrial strips were excised from the upper edge of the lower uterine segment. Strips were quick frozen in liquid nitrogen and stored at -80° C until assayed. <sup>125</sup>I-labeled iodocyanopindolol was obtained from New England Nuclear and had a specific activity of 2200 Ci/mmol. Stereoisomers of propranolol were gifts of Ayerst. We wish to thank J. Polanski, M.D., for the specific  $\beta_2$ -antagonist IPS 339.<sup>3</sup> All other chemicals and drugs were of the highest grade commercially available.

**Tissue preparation.** Myometrial strips were dissected

from serosa, blood clot, and endometrium and then minced and homogenized on ice in a Tissumizer (Tekmar, Cincinnati, Ohio) three times at the highest setting for 15 seconds with 1-minute cooling intervals. The homogenate was vacuum filtered through two layers of cheesecloth, then centrifuged at 800 × g for 15 minutes. The supernatant was recentrifuged at 25000 × g for 15 minutes. The resultant pellet was resuspended in Trizma base: 50 mmol/L Tris(hydroxymethyl)aminomethane (Sigma Chemical), 4 mmol/L of magnesium, pH 7.4 (Tris-Mg), at 30° C. Preparations were then stored at -80° C until assayed. Binding of iodocyanopindolol was similar in fresh particulates and in those stored for up to 4 months. Protein concentrations were determined by the method of Bradford,<sup>1</sup> with bovine serum albumin as the standard.

**Assay.** Assays were performed in 0.25 ml of 50 mmol/L Tris at pH 7.4 which contained particulate at 0.24 mg/ml of protein, 4 mmol/L Mg, iodocyanopindolol at concentrations from 50 to 500 pmol/L with or without 0.1 mmol/L of isoproterenol. Samples were incubated at 30° C for 60 minutes. The incubation was stopped by addition of 5 ml of Tris-Mg at 4° C and immediate filtering through Whatman GF/C filters. An additional 15 ml of cold Tris-Mg was used to wash filters while under low vacuum (1 ml/sec). Filters were dried under high vacuum and then counted in an Auto-Gamma Spectrometer (Packard) at a machine efficiency of 67%. Competition experiments with the different adrenergic agonists and antagonists were performed in a similar fashion with a constant radioligand concentration of 50 pmol/L and different concentrations of competitor.

Saturation experiments were analyzed by a nonlinear curve fitting program prepared for a Hewlett-Packard 9825B computer.<sup>5</sup> This program analyzes bound radioligand as a function of free radioligand. The data



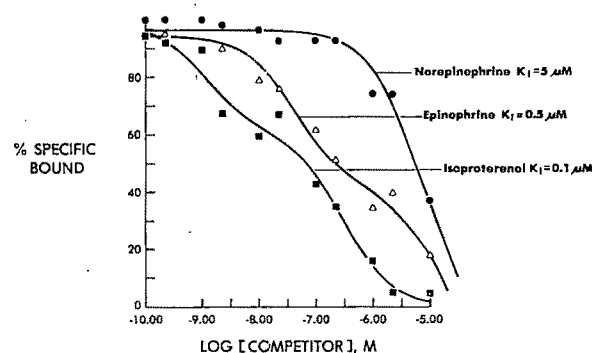
**Fig. 1.** Scatchard saturation analysis of total binding of iodocyanopindolol. Concentration of iodocyanopindolol from 6 to 200 pmol/L were incubated with myometrial particulate (final concentration, 0.24 mg/ml) for 60 minutes at 30° C. Data are experimental data. The curve through these points is that determined by the parameters from the computer-assisted analysis of bound versus free iodocyanopindolol. The straight lines are the resolved components of this curve, indicating a saturable and nonsaturable component at these concentrations of iodocyanopindolol.  $B_{max} = 16$  fmol/mg protein,  $K_d = 20$  pmol/L.

are arrayed as a Scatchard plot with use of parameters determined with the curve fitting program. In preliminary experiments we compared the computer estimate of highest affinity binding with specific binding determined as that competed by 0.1 mmol/L of 1-isoproterenol. The receptor concentration (high affinity binding,  $32 \pm 5$  fmol/mg of protein; specific binding,  $34 \pm 5$  fmol/mg of protein) and dissociation constant (high affinity binding,  $17 \pm 7$  pmol/L; specific binding,  $18 \pm 4$  pmol/L) determined by these techniques were not different. To increase precision and to conserve tissue, we determined binding parameters by analysis of highest affinity binding. Specific binding in competition experiments was also analyzed by an iterative curve fitting program to determine the concentration of competitor-reducing iodocyanopindolol binding by 50% (150). The inhibition constant ( $K_i$ ) was determined for each competing adrenergic agent by the relationship described by Cheng and Prusoff<sup>6</sup>:  $K_i = 150/(1 + L/K_d)$ , where  $L$  is the concentration of iodocyanopindolol used for the assay and  $K_d$  is the dissociation constant of iodocyanopindolol for its binding sites. Specific binding in these experiments was determined as that competed by 0.1 mmol/L of isoproterenol.

Statistical analyses were performed with one-way analysis of variance or unpaired Student's  $t$  test. Data are presented as the mean  $\pm$  SEM.

## Results

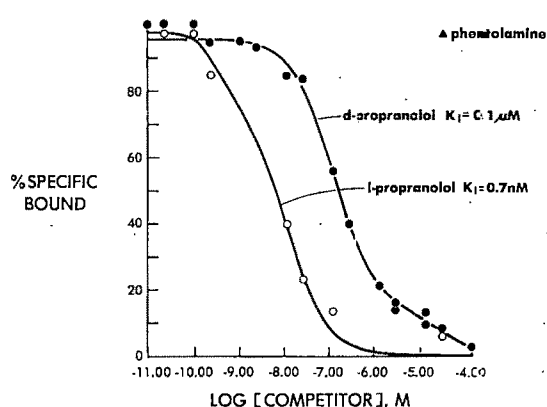
**Characterization of iodocyanopindolol as binding to the  $\beta$ -receptor.** Binding of iodocyanopindolol to hu-



**Fig. 2.** Order of potency of agonist affinities competing for iodocyanopindolol binding sites. Iodocyanopindolol, 50 pmol/L, was incubated with myometrial particulates (final protein concentration, 0.24 mg/ml) and increasing concentrations of agonists. Binding is expressed as a percentage of specific binding.  $K_i$  of isoproterenol, 0.1  $\mu$ mol/L;  $K_i$  of epinephrine, 0.5  $\mu$ mol/L;  $K_i$  of norepinephrine, 5  $\mu$ mol/L. These  $K_i$ s are determined by the mathematical model predicting that these adrenergic agonists interact with the iodocyanopindolol binding site with a single affinity; for both isoproterenol and epinephrine, the data were best fit by the interaction of these agonists with two classes of sites (isoproterenol:  $K_h$  of 0.8 nmol/L,  $K_l$  of 0.2  $\mu$ m,  $R_h/R_l$ ).

man myometrial preparations was high affinity ( $K_d$ ,  $17 \pm 7$  pmol/L) and saturable (Fig. 1). Equilibrium time at 50 pmol/L of iodocyanopindolol was 45 minutes. Adrenergic agonists competed with iodocyanopindolol in a manner compatible with  $\beta_2$ -adrenergic potencies: isoproterenol ( $K_i$ , 0.1  $\mu$ mol/L) > epinephrine ( $K_i$ , 0.5  $\mu$ mol/L) > norepinephrine ( $K_i$ , 5  $\mu$ mol/L)<sup>7</sup> (Fig. 2). These values are the  $K_i$ s determined by the mathematical model predicting that these adrenergic agonists interact with the iodocyanopindolol binding site with a single affinity. For both isoproterenol and epinephrine the data were best fit by the interaction of these agonists with two classes of sites. Data for isoproterenol were  $K_h = 0.8$  nmol/L,  $K_l = 0.2$   $\mu$ mol/L,  $R_h/R_l = 42\%$ , and for epinephrine were  $K_h = 14$  nmol/L,  $K_l = 3.3$   $\mu$ mol/L,  $R_h/R_l = 55\%$  (where  $K_h$  is the  $K_i$  for the high affinity binding site,  $K_l$  is the  $K_i$  for the low affinity binding site,  $R_h$  is the receptor concentration for the high affinity binding site, and  $R_l$  is the total receptor concentration).

The  $\beta$ -adrenergic antagonist propranolol was a much more effective competitor than the  $\alpha$ -adrenergic antagonist phentolamine. The competition of propranolol was with a single class of sites and was stereoselective. The pharmacologically active stereoisomer 1-propranolol was 100 times as potent a competitor as the inactive d-isomer (Fig. 3). The  $\beta_2$  selective adrenergic antagonist IPS 339 competed for 72% of the iodocyanopindolol binding sites with an affinity consistent with  $\beta_2$  interactions ( $K_i$ , 2 nmol/L), whereas 28% of the iodocyanopindolol binding sites competed with an af-



**Fig. 3.** Stereoselective competition for idocyanopindolol binding sites. Idocyanopindolol, 50 pmol/L, was incubated with myometrial particulate (final concentration, 0.24 mg/ml) and increasing concentration of propranolol. Binding is expressed as percentage of specific binding. The  $-$  stereoisomer of propranolol was 100 times as potent as the  $+$  stereoisomer. ( $K_i$  of  $-$  propranolol = 0.7 nmol/L,  $K_i$  of  $+$  propranolol = 1  $\mu$ mol/L, 42%; epinephrine  $K_b$  = 14 nmol/L,  $K_i$  = 3.3  $\mu$ mol/L,  $R_b/R_i$  = 55%).

finity consistent with  $\beta_1$  interactions ( $K_i$ , 0.4  $\mu$ mol/L) (Fig. 4).

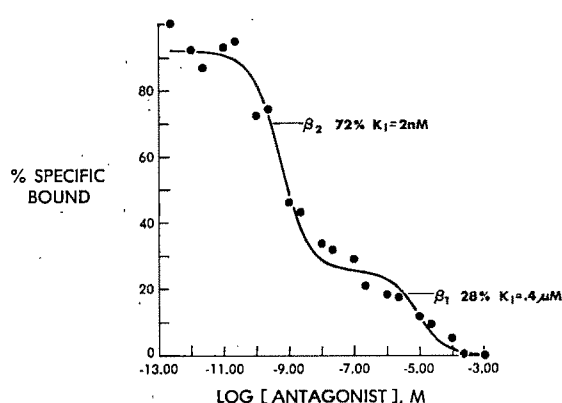
**$\beta$ -Receptor concentrations.** To investigate the possible role of  $\beta$ -adrenergic receptors in parturition, we compared the concentrations of  $\beta$ -receptors from women at term in labor or before labor (Fig. 5). Table I summarizes the indications for cesarean section in term pregnant women. The  $\beta$ -receptor concentrations in myometrium from women at term and in labor ( $25 \pm 4$  fmol/mg of protein) was not different from  $\beta$ -receptor concentrations from women at term before labor ( $32 \pm 5$  fmol/mg of protein). Dissociation constants were also not different.

Table II summarizes both the data and the clinical circumstances for all women delivered prematurely.  $\beta$ -Receptor concentrations in myometrial preparations obtained from women in preterm labor ( $20 \pm 4$  fmol/mg of protein) were not significantly different from  $\beta$ -receptor concentrations in preterm women not in labor ( $27 \pm 9$  fmol/mg of protein) nor were they different from  $\beta$ -receptor concentration at term (Fig. 6).

Isoproterenol competition for idocyanopindolol binding in myometrium was used as a measure of efficacy. In other systems, high affinity agonist binding indicates interaction of the receptor with a regulatory protein. This is linearly related to the efficacy of the agonist.<sup>8</sup> The percent of high affinity binding in women at term who were in labor (57% high affinity binding), before labor (56%), or in preterm labor (57%) was not different, indicating no difference in agonist efficacy.

# Comment

<sup>125</sup>I-labeled idocyanopindolol bound to human myometrium in a manner compatible with binding to the



**Fig. 4.** Competition of IPS 339 (the  $\beta_2$  selective antagonist) for idocyanopindolol binding sites. Idocyanopindolol, 50 pmol/L, was incubated with myometrial particulate (final protein concentration, 0.24 mg/ml) and the indicated concentrations of IPS 339. Binding is expressed as a percent specific binding. The curve is the curve of best fit as defined by the iterative, nonlinear curve fitting program and was determined by the following parameters:  $K_b$ , 2 nmol/L,  $\%R_b$ , 72%;  $K_i$ , 0.4  $\mu$ mol/L,  $\%R_i$ , 28%.

$\beta$ -receptor. Idocyanopindolol bound with high affinity and was saturable. It was both  $\beta$ -receptor specific and stereoselective. The competition of isoproterenol, epinephrine, and norepinephrine for the idocyanopindolol binding site demonstrated  $\beta_2$ -adrenergic potencies. Consistent with this, competition with the selective  $\beta_2$ -antagonist IPS 339 indicated that 72% of the receptors present in the myometrial particulate were of the  $\beta_2$ -subtype. Results with idocyanopindolol are comparable to our previously reported experience with the radioligand tritiated dihydroalprenolol.<sup>9</sup> Receptor concentrations determined from term pregnant myometrium when assayed with dihydroalprenolol were 70 fmol/mg of protein. Agonist competition with isoproterenol ( $K_i$ , 0.12  $\mu$ mol/L), epinephrine ( $K_i$ , 1.1  $\mu$ mol/L), and norepinephrine ( $K_i$ , 50  $\mu$ mol/L) mirrored  $\beta_2$ -adrenergic potencies and were similar to our results with idocyanopindolol. Studies with dihydroalprenolol and the specific  $\beta_2$ -agonist zinterol indicated that 87% of the receptors present in the tissue assayed with dihydroalprenolol were of the  $\beta_2$ -subtype. The difference between 87% and the 72% determined with the idocyanopindolol is similar within the discriminatory capacity of this data analysis technique. We found use of idocyanopindolol preferable to dihydroalprenolol because the high affinity and specific activity of idocyanopindolol permitted the assay of smaller concentrations of protein (1.25 mg/ml of dihydroalprenolol versus 0.3 mg/ml of idocyanopindolol). This is particularly advantageous with the limited amounts of human tissue usually available.

Our results from competition experiments in term pregnant uterus indicated that data obtained with both isoproterenol and epinephrine were best described by



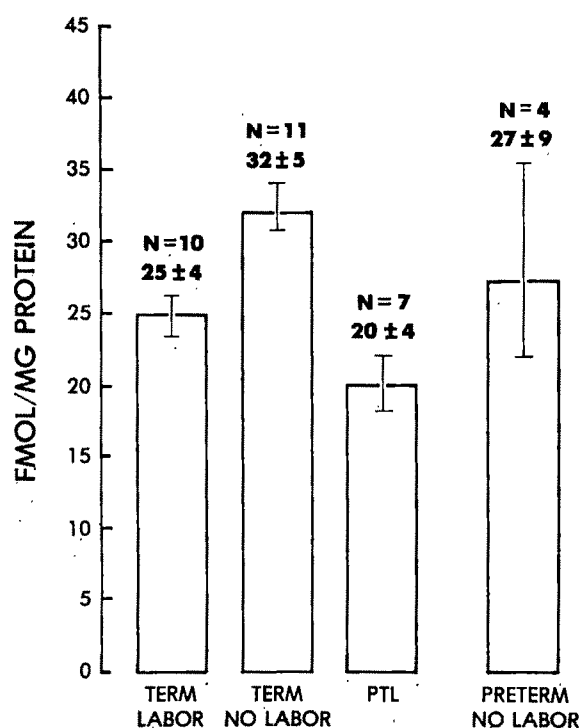


Fig. 5. Concentration of  $\beta$ -receptors. Myometrial particulate from pregnant women at term in labor or before labor or between 28 and 34 weeks of gestation in preterm labor or before labor was incubated for 60 minutes with iodocyanopindolol, 6 to 200  $\mu$ mol/L at 37° C. Receptor total is expressed in fmol/mg of protein. All results are the mean  $\pm$  SEM.

a model in which these agonists competed for iodocyanopindolol binding sites with two affinities. The ratio of the high and low affinity interactions and percentage of high affinity binding was similar for these two agonists (isoproterenol,  $K_i/K_h = 272$ ,  $\%R_h = 42\%$ , and epinephrine,  $K_i/K_h = 225$ ,  $\%R_h = 55\%$ ). In contrast, norepinephrine competition for the iodocyanopindolol binding sites was best described by a model in which norepinephrine competed with a single affinity (Fig. 2). (In an attempt to address affinity independent of efficacy, we have compared the  $K_i$  for isoproterenol and for epinephrine with that of norepinephrine as the  $K_i$  determined by one site.)

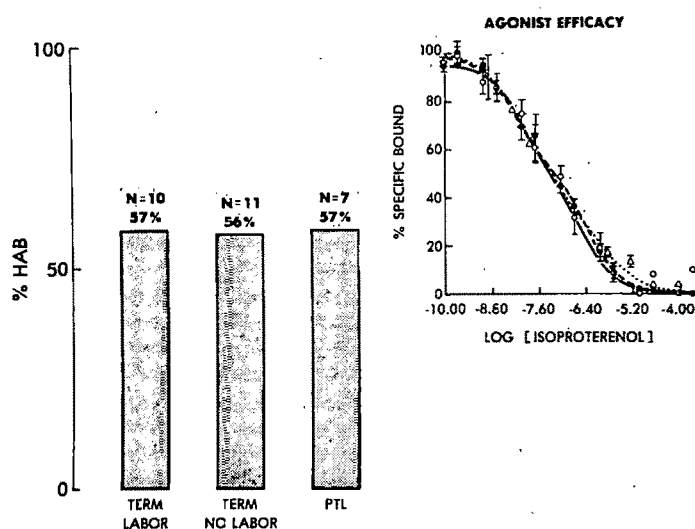
In other systems, such as the frog erythrocyte, a larger percentage of high affinity binding ( $\%R_h$ ) and an increase in the ratio of low to high affinity binding ( $K_i/K_h$ ) is related to a greater functional efficacy of an agonist. In frog erythrocytes, the efficacy, defined as the ability to activate adenylate cyclase at full receptor occupancy, of several adrenergic agonists was linearly related to either the ratio of the two affinity forms ( $K_i/K_h$ ) or the percentage of sites with which the agonists interacted with high affinity, ( $\%R_h$ ).<sup>8</sup> In this system, therefore, these parameters directly corresponded to a

pharmacologic efficacy of the agonist. A comparison of the relative  $K_i/K_h$  found in our studies with human myometrium (isoproterenol,  $K_i/K_h = 272$ ,  $\%R_h = 42$ , and epinephrine,  $K_i/K_h = 225$ ,  $\%R_h = 55$ ) suggests that though the affinity of these two agonists for the  $\beta$ -receptor is quite different, they have similar efficacy. The absence of high affinity norepinephrine binding suggests that the different potencies of isoproterenol, epinephrine, and norepinephrine in myometrium may relate to a different efficacy as well as affinity of these agonists.

The concentration of  $\beta$ -receptors in myometrial particulate preparations from women at term in labor ( $25 \pm 4$  fmol/mg of protein) was not different from myometrial particulate preparations from women at term before labor ( $32 \pm 5$  fmol/mg protein). Even though  $\beta$ -receptor concentrations were similar in term pregnancy, with or without labor, it is possible that mechanisms for term and preterm labor are dissimilar. To test this, myometrium from patients between 28 and 34 weeks of gestation was assayed. There was no significant difference in the concentration of  $\beta$ -receptors between term and preterm gestations. Similarly, there was no statistical difference in  $\beta$ -receptor concentration between any of the preterm gestations when analyzed by gestational age. In clinical studies, prolonged treatment with  $\beta$ -sympathomimetics appears to have decreased efficacy over time and in rabbit lung, glucocorticoids increase the concentration of  $\beta$ -receptors.<sup>10</sup> Therefore, our results in preterm women may be confounded by the fact that at the time of preterm delivery they were frequently treated with  $\beta$ -agonists and/or glucocorticoids. The potential "down regulation" of  $\beta_2$ -receptors that may occur with prolonged use of  $\beta$ -sympathomimetics would be expected to cause a decrease in the concentration of  $\beta_2$ -receptors. Thus although we cannot eliminate the confounding effects of the  $\beta$ -agonist, this treatment would be predicted to result in reduced receptor concentration in the laboring patients, since this group includes the majority of patients receiving tocolytic therapy. Since our findings indicate similar receptor concentration in myometrium from preterm patients with or without labor, it is unlikely that decreased  $\beta$ -receptor concentration is an important contributor to preterm parturition.

There is evidence that the efficacy of the  $\beta$ -receptor for a given adrenergic agonist is not static but is subject to hormonal regulation.<sup>11</sup> To test whether efficacy of  $\beta$ -agonist receptor interaction might decrease in labor and perhaps be causally related to the onset of parturition, we measured  $\%R_h$  and  $K_i/K_h$  in the different preparations.

The percentage of high affinity binding sites identified by isoproterenol in particulate myometrium from



**Fig. 6.** Agonist efficacy expressed as percentage high affinity agonist binding. Myometrial particulate from pregnant women at term in labor (57%), before labor (56%) or between 28 to 34 weeks' gestation in preterm labor (57%) was incubated with increasing concentrations of isoproterenol and competed with iodocyanopindolol, 50 pmol/L (*upper right corner*). The percent of high affinity binding (*HAB*), a measure of efficacy, was not different in any of the groups studied (*bar graph*).

patients at term in labor (57%), before labor (56%), or in preterm labor (57%) indicated no difference in competition of isoproterenol for the high affinity binding site. The similar  $\%R_h$  found in term and preterm myometrium in the presence or absence of labor suggests that there is no change in the efficacy of the  $\beta$ -receptor for  $\beta$ -agonists with the onset of labor.

Our hypothesis was that a reduction in the concentration of  $\beta$ -receptors may be related to the initiation of parturition. Our data for receptor concentrations from term and preterm pregnant women indicated no significant change in  $\beta$ -receptor concentrations with labor. Several explanations may exist for this finding. First, the concentrations of  $\beta$ -adrenergic receptors in the lower uterine segment from which our samples were taken may not be representative of  $\beta$ -receptor concentrations in the more relevant contractile portion of the uterus (especially when expressed as a function of particulate protein). This has, in fact, been reported for human myometrial oxytocin receptors. However, previous work from our laboratory has indicated that the concentration of  $\beta$ -receptors is similar in all areas of the uterus. Second, the function of the  $\beta$ -receptors may differ between the more "inactive" lower uterine segment (from which our samples were obtained) and the more contractile fundal portion. Third, alterations in myometrial  $\beta$ -adrenoreceptors may have no role in the initiation of term parturition. Our findings of minimal alteration of  $\beta$ -adrenoreceptor concentration in spite of the changing hormonal milieu accompanying the onset of parturition are consistent with previous studies from our laboratory on the influence of gonadal

steroids on the concentration of  $\beta$ -adrenoreceptors in rabbit uterus. In these studies we found that  $\beta$ -receptor concentration was similar with either estrogen treatment or sequential estrogen/progesterone treatment.<sup>1</sup> It is possible that the  $\beta$ -adrenoreceptor, unlike the  $\alpha$ -receptor, is not regulated by estrogens.

In summary, we found  $^{125}$ I-labeled iodocyanopindolol binding a sensitive and accurate radioligand for studies of human myometrial  $\beta$ -receptors. It is preferable to dihydroalprenolol because it enables assay of smaller quantities of tissue with equal accuracy. Our findings do not support the hypothesis that a decreasing concentration of myometrial  $\beta$ -receptors is necessary for the onset of term parturition. It also does not appear that a decreased concentration of  $\beta$ -receptors is associated with preterm labor or that myometrial  $\beta$ -receptor concentration changes with advancing gestation. These latter conclusions must be tempered by the confounding effects of the heterogeneity of the preterm patients examined, particularly when preterm labor patients received the  $\beta$ -agonist ritodrine and/or glucocorticoids. Similarly, examination of the  $\beta$ -adrenergic receptor in myometrial preparations by competition of isoproterenol for iodocyanopindolol binding indicated no change in high affinity binding and suggests that there is no change in efficacy of receptor agonist interactions with the onset of labor.

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## Biochemical and histologic effects of sequential estrogen/progestin therapy on the endometrium of postmenopausal women

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Medroxyprogesterone acetate in doses of 10, 5, and 2.5 mg was administered sequentially to three groups of postmenopausal women receiving 0.3 mg, 0.625 mg, and 1.25 mg of conjugated equine estrogens, respectively. Serial endometrial biopsies were performed on these women before therapy, during estrogen therapy alone, and during sequential estrogen-progestin therapy. Endometrial histology and estrogen receptor concentrations were assessed. A linear increase of cytosolic estrogen receptor concentration occurred over the dosage range of conjugated equine estrogen. When medroxyprogesterone acetate was added to the estrogen therapy, the concentrations of estrogen receptors fell. Within the groups of women receiving 0.3 mg and 0.625 mg of conjugated equine estrogen, all doses of medroxyprogesterone acetate were equally effective in reducing the levels of cytosolic receptor to pretreatment levels. However, at the conjugated equine estrogen dose of 1.25 mg, only 5 mg and 10 mg doses were effective in reducing the cytosolic receptor concentration to pretreatment levels. Histologically, little effect was observed from the lowest doses of either drug. However, even though 5 and 10 mg of medroxyprogesterone acetate were identical biochemically, the 10 mg dose was the only one producing a homogeneous, secretory pattern within the endometrium. (*Am J Obstet Gynecol* 1986;154:456-61.)

**Key words:** Postmenopausal endometrium, hormone replacement

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The administration of estrogens to postmenopausal, estrogen-deficient women is associated with the prevention of osteoporosis and the reduction of the risk of hip fracture.<sup>1, 2</sup> However, epidemiologic studies indicate that unopposed estrogen therapy in these women increases their risk of developing endometrial

cancer.<sup>3-5</sup> Studies by Paterson et al.<sup>6</sup> suggest that abnormalities of endometrial histology induced by estrogen therapy are both dose and duration dependent but can be antagonized by concurrent administration of a progestin. Other authors have reported that the addition of a progestin to postmenopausal estrogen replacement therapy apparently reduces the risk of endometrial cancer.<sup>7,8</sup> These studies suggest that "maximal" protection is obtained by using a progestin for 10 or more days of the treatment cycle.

The 19-nortestosterone compounds have been shown by Whitehead et al.<sup>9</sup> to be an effective antagonist of nuclear accumulation of estrogen receptor and cellular mitotic activity. However, the 19-nortestosterones appear to reverse the beneficial elevation of high-density lipoproteins occurring from estrogen administration, while medroxyprogesterone acetate is much less potent in this regard.<sup>9,10</sup> For this reason a decision was made to evaluate varying combinations of conjugated equine estrogens and medroxyprogesterone acetate to determine whether a dose-response relationship could be observed for changes in endometrial histologic conditions and estrogen receptor concentration.

#### Material and methods

Thirteen women agreed to participate in the study. The average age of the subjects was 57; all had undergone a natural menopause; all were >2 years from their last menstrual period, and all were within 20% of their ideal body weight. Complete history and physical examinations were unremarkable. Before the initiation of hormone therapy, baseline serum estrone and estradiol levels were determined and a pretreatment endometrial biopsy obtained. The biopsies were performed with either a Rock-Garcia or Novak curette with the patient under paracervical block anesthesia. Because the concentrations of cytosolic estrogen receptor have been reported to be lower in the lower uterine segment than in the fundus,<sup>11</sup> every effort was made to obtain all biopsies from the anterior fundal regions of the uterine cavity. Two biopsies per treatment cycle were obtained to assure adequate tissue for both histologic review and estrogen receptor determinations.

Conjugated equine estrogens were administered for 25 days per treatment cycle to three women in a dose of 0.3 mg, to five women in a dose of 0.625 mg, and to five women in a dose of 1.25 mg. There were a total of four treatment cycles, and each treatment cycle was separated by a 4-week rest interval. The first treatment cycle consisted of estrogen alone. The next three treatment cycles consisted of the same dose of estrogen but in combination with increasing doses of medroxyprogesterone acetate (2.5 mg, 5 mg, and 10 mg) administered on treatment days 15 to 25.

**Table I.** The morphologic changes in the glands and stroma evaluated after progestin administration to estrogen-primed endometrium

Category	Method of evaluation
Gland epithelial height ( $\mu$ m)	Average height of endometrial glands measured from basement membrane to apex of plasma membrane
Gland diameter ( $\mu$ m)	Average diameter of endometrial glands measured from basement membrane
Glands showing secretion (%)	Percent of glands in the zona functionalis showing luminal fluid; physiologic endometria of 6-7 days' postovulation show that 80% to 100% of glands contain fluid and the late proliferative endometria show that 0 to 10% of glands contain intraluminal fluid
Quality of secretion (+)	Includes the amount of luminal secretory fluid, number of apical buds, and degree of gland tortuosity; changes (4+) show features seen in physiologic endometria at 6-7 days' postovulation and 0 is seen in late proliferative endometria
Pseudodecidual stroma (+)	Endometrial stromal cells that are oval or round, show increased cell volume, eosinophilic cytoplasm, and vesicular nuclei; stromal changes (4+) are seen in physiologic endometria at 11 to 12 days' postovulation and 0 is found before the seventh day post-ovulation
Subnuclear vacuoles (%)	Percent of endometrial gland cells showing a clear vacuole between the nucleus and basement membrane

On the twenty-fifth day of a treatment cycle an endometrial biopsy was obtained. In addition, serum was drawn for estrone and estradiol determinations. For histologic evaluation the biopsy tissue was placed into formalin, dehydrated, and blocked in paraffin sections (all of the slides were read by the same individual, D. L. M.). A system was devised for grading progestational change in the tissue (see Table I). To test whether the 4-week interval between treatment cycles was sufficient for return of receptor levels and histology to the pretreatment state, an endometrial biopsy was obtained at the end of the first rest interval. Additionally, a final biopsy was obtained 4 weeks after the last treatment cycle to document the normalcy of the endometrium before the subjects were discharged from



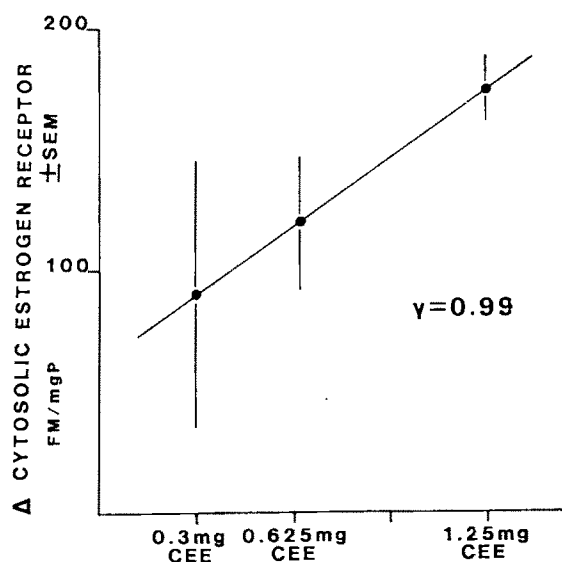


Fig. 1. The relationship between the dose of conjugated equine estrogens and the average increase in cytosolic estrogen receptor concentrations from their matched pretreatment levels.

the study. No subject discontinued the study before its completion.

For estrogen receptor analysis the endometrial biopsy tissues were placed into an ice-cold, isotonic buffer solution (10 mmol/L of Tris, 1.5 mmol/L of ethylenediaminetetraacetic acid, 12 mmol/L of thioglycerol, and 10% glycerol) and were frozen at  $-85^{\circ}\text{C}$  until assayed (within 1 week). The estrogen receptor studies were performed as described in a previous publication.<sup>12</sup> The tissue obtained was placed into 2 ml of cold buffer and homogenized with a Tekmar tissue homogenizer. The suspension was then placed into a SW55 rotor (Beckman Instruments, Fullerton, California) and centrifuged at  $100,000 \times g$  in a Beckman/Spinco L8-80 ultracentrifuge for 1 hour. The supernatant from this spin constituted the "cytosol" used for cytosolic estrogen receptor concentration. The pellet was washed by resuspending it in buffer (times two) and then repelleting it by centrifugation at  $10,000 \times g$ . This pellet constituted the "crude nuclear pellet," which was used for measurement of nuclear estrogen receptor concentration. This pellet was resuspended in 1.6 ml of buffer. The minimal amount of tissue required for measurement of cytosolic receptor was 20 mg, while 30 mg of tissue was the lower limit for the nuclear assay. The lower limit of allowable protein concentration for this assay was 0.05 to 0.1 mg/ml measured by the Coomassie blue method (BioRad Labs, Richmond, California). A five-point Scatchard assay was performed with a concentration range for the radiolabeled estradiol (specific activity = 100 Ci/mmol) of 0.05 to 2 nmol/L. A 200-fold molar excess of unlabeled diethylstilbestrol was

used in a parallel series of tubes to distinguish specific from total binding. The incubation volume was 0.3 ml, which included the 0.1 ml aliquots of the cytosol or nucleosol preparation. The duration of incubation for the cytosol receptor measurements was overnight at a temperature of  $4^{\circ}\text{C}$ . This temperature selects for the measurement of unoccupied binding sites.

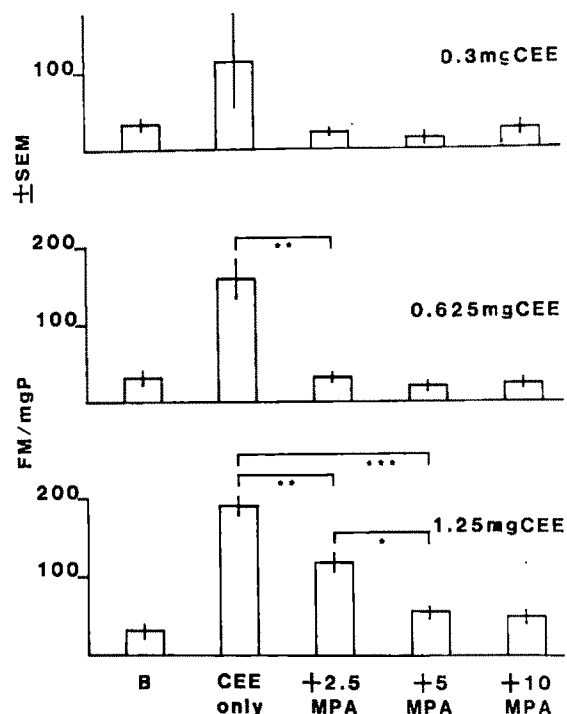
The conditions for the nuclear assay were similar except that the incubation conditions included a 30-minute incubation at  $30^{\circ}\text{C}$  to stimulate the exchange of radiolabeled steroid for the endogenous steroid occupying the nuclear receptor. Bound and free hormone were separated by adding 0.2 ml of hydroxylapatite slurry to each receptor solution.<sup>12</sup> The data were analyzed according to the method of Scatchard.<sup>13</sup> The limit of detectable receptor in this assay was 4 fmol/mg of protein. The term "cytosolic" in the context of its use in this report refers to the receptor population that is soluble and not tightly bound to the crude nuclear pellet/chromatin fraction. It does not preclude the recent observation that the *in vivo* location of all estrogen receptor populations may be in the nuclear compartment.<sup>14</sup>

Analysis of variance by means of the Minitab statistical program package (versions 81.1 and 82.1, Pennsylvania State University) was used to evaluate the relationship of changes in receptor concentration resulting from perturbations of estrogen-progestin therapy. The morphologic criteria were analyzed by the paired Student's *t* test. Correlation coefficients were determined by regression analysis.

## Results

**Pretreatment receptor levels.** Before treatment, the average serum concentrations of estrone and estradiol were  $25 \pm 17$  pg/ml ( $\pm 1$  SD) and  $10 \pm 4.5$  pg/ml, respectively. The average concentration of cytosolic estrogen receptor in these pretreatment specimens was  $33.5 \pm 28$  fm/mgp ( $n = 13$ ). There was detectable cytosolic receptor in all but two of the cytosols. The average concentration of nuclear estrogen receptor in the pre-treatment tissues was 48.7 fmol of protein with 10 of the 13 biopsies revealing no detectable receptor levels (the protein content of the crude nuclear pellet exceeded the lower limits of the assay in all but two of the specimens). In comparison, the average cytosolic and nuclear receptor levels in the biopsies obtained at the end of the first rest cycle were  $32 \pm 25$  and  $50 \pm 37$  fmol of protein, respectively. Five of 12 of these biopsies failed to demonstrate measurable nuclear receptor.

**Receptor levels during therapy.** Measurement of the difference between the baseline value of an individual patient's cytosolic receptor concentration and her level in the estrogen-alone treatment cycle showed a relationship between the dose of estrogen given and the

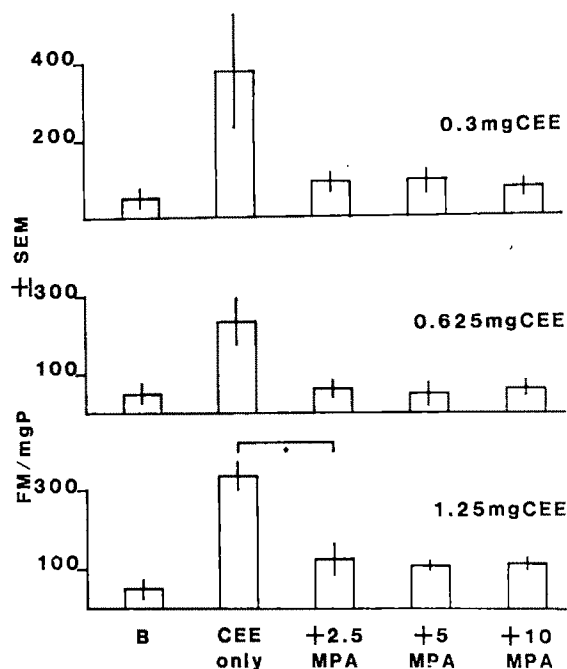


**Fig. 2.** The concentrations of cytosolic estrogen receptor in the three groups of postmenopausal women receiving no therapy (B), estrogen only (CEE), and estrogen plus various dosages of medroxyprogesterone acetate (MPA). \* =  $p < 0.025$ , \*\* $p < 0.001$ , \*\*\* =  $p < 0.001$ .

amount of estrogen receptor measured (see Fig. 1). In the group of women receiving 0.3 mg of conjugated estrogens, the average increase of estrogen receptor in the cytosol was 89.5 fmol of protein ( $n = 3$ ). The average increase in receptor concentration was 119.8 fmol of protein in the group receiving 0.625 mg of conjugated estrogens ( $n = 5$ ). The average increase in cytosolic receptor concentration was 174 fmol of protein in the group receiving 1.25 mg of conjugated estrogens ( $n = 5$ ). This increase was linear with a correlation coefficient of 0.99. Because of the high variability observed in the nuclear receptor concentrations, particularly at the lowest estrogen dose, no relationship was observed (see Fig. 3) between the dose of estrogen administered and the amount of nuclear receptor.

The addition of 2.5 mg of medroxyprogesterone acetate to the estrogen treatment cycles resulted in a suppression of cytosolic estrogen receptor to levels seen before treatment in women receiving 0.3 and 0.625 mg of conjugated estrogens but not in those women receiving 1.25 mg of conjugated estrogens (see Fig. 2). At the medroxyprogesterone doses of 5 and 10 mg, the progestin was capable of suppressing the cytosolic receptor levels in all three estrogen treatment groups.

When pretreatment and posttreatment levels of nuclear estrogen receptor were compared (Fig. 3), similar



**Fig. 3.** The concentrations of nuclear estrogen receptor in the three groups of postmenopausal women receiving no therapy (B), estrogen only (CEE), and estrogen plus various dosages of medroxyprogesterone acetate (MPA). \* =  $p < 0.001$ .

suppression of estrogen-stimulated increases of nuclear receptor was seen at all medroxyprogesterone acetate doses in all three estrogen treatment groups.

**Histologic findings.** Review of the histologic patterns of pretreatment biopsies revealed atrophic endometrium in all cases as did all of the biopsies performed 4 weeks after the last treatment cycle. Only minimal estrogenic change was observed in the group receiving 0.3 mg of estrogen alone, whereas a marked estrogenic stimulation of the endometrium occurred with administration of 0.625 and 1.25 mg of conjugated estrogen for 25 days. The effects of the two higher estrogen doses were nearly indistinguishable.

When medroxyprogesterone acetate was added to the estrogen therapy, the morphologic effects varied with the dose administered. None of the endometrial biopsies taken from women receiving 2.5 mg of the progestin demonstrated a progestational response. Because of their biochemical similarity, the 5 mg and 10 mg of medroxyprogesterone acetate treatment cycles were compared for progesterone effect by the parameters presented previously. In three of the patients treated with 0.625 mg of estrogen there was sufficient material to evaluate all of the morphometric parameters (Table II). Comparison of the average values of the parameters showed that 10 mg of medroxyprogesterone acetate promoted a greater progestational response than did the 5 mg dose.

For the subjects receiving 1.25 mg of estrogen, com-

**Table II.** Histologic comparison of 5 and 10 mg of medroxyprogesterone acetate on 0.625 mg of conjugated estrogen (n = 3) ( $\pm$  SD):

	5 mg	10 mg
Gland epithelial height ( $\mu$ m)	17.3 $\pm$ 4.0	23.3 $\pm$ 7.6
Gland diameter ( $\mu$ m)	63.3 $\pm$ 20.8	105 $\pm$ 35
Glands showing secretion (%)	6.7 $\pm$ 5.8*	50 $\pm$ 14.1*
Quality of secretion (+)	0.5 $\pm$ 0.5	2.16 $\pm$ 1.8
Pseudodecidual stroma (+)	0 $\pm$ 0	1.13 $\pm$ 1.1
Subnuclear vacuoles (%)	5 $\pm$ 7.1	1 $\pm$ 1.4

\*p &lt; 0.05.

parison of 5 mg and 10 mg of progestin again demonstrated a greater progestational effect by the higher dose of progestin (Table III). More importantly, not only was there a greater overall progestational effect observed within the groups receiving 10 mg of medroxyprogesterone acetate, but the higher dose resulted in more homogeneous, secretory changes in the stroma and glands than were apparent with the 5 mg dose.

#### Comment

The use of conjugated equine estrogens in this population of postmenopausal women resulted in a linear increase in the concentration of endometrial cytosolic estrogen receptors. This dose-dependent effect was also seen to a lesser extent when the morphologic condition of the endometrium was examined. A relationship between estrogen dose and biologic effect has been observed by Paterson et al.,<sup>6</sup> who described a dose and duration effect of estrogens on abnormal endometrial form and structure. It has been previously reported that estrogen increases the concentration of its own receptor population.<sup>15</sup> A drug dose-response relationship was not discernible for nuclear estrogen receptor concentration and estrogen dosage. This primarily resulted from the marked variability and small number of samples in the 0.3 mg of estrogen dose group.

Evaluation of the cytosolic estrogen receptor data alone would suggest that 2.5 mg of medroxyprogesterone acetate administered for 10+ days of an estrogen treatment cycle would antagonize the estrogenic effects of 0.3 mg and 0.625 mg of conjugated equine estrogens, but not those of 1.25 mg. On the other hand, it appears from the cytosolic receptor data that 5 mg of medroxyprogesterone acetate works as well as 10 mg as an estrogen antagonist within the dose range of conjugated estrogens examined in this study.

The nuclear estrogen receptor concentrations were also decreased by medroxyprogesterone acetate administration. The inhibition of estrogen-induced increases in nuclear estrogen receptor concentration by

**Table III.** Histologic comparison of 5 and 10 mg of medroxyprogesterone acetate on 1.25 mg of conjugated estrogen (n = 4) ( $\pm$  SD):

	5 mg	10 mg
Gland epithelial height ( $\mu$ m)	23.5 $\pm$ 4.4*	35.7 $\pm$ 9.9*
Gland diameter ( $\mu$ m)	91.2 $\pm$ 27.5	127.5 $\pm$ 35.7
Glands showing secretion (%)	42.5 $\pm$ 27.2†	88.75 $\pm$ 9.5†
Quality of secretion (+)	2 $\pm$ 1.1‡	3.5 $\pm$ 0.6‡
Pseudodecidual stroma (+)	0.875 $\pm$ 1.4	2.5 $\pm$ 1.0
Subnuclear vacuoles (%)	13.7 $\pm$ 7.5	3.75 $\pm$ 1.5

\*p &lt; 0.05.

†p &lt; 0.01.

‡p &lt; 0.025.

progesterone is a well-accepted mechanism of progesterone antagonism of estrogen action.<sup>16</sup> Seaver et al.<sup>17</sup> observed that administration of progesterone results in a reduction in estrogen-stimulated transcription and thus of messenger-RNA synthesis. Note that even though similar nuclear concentrations of estrogen receptor were present with the different progestin doses within the 1.25 mg estrogen group, statistically significant different levels of receptor were observed in the cytosolic compartment. This is because mechanisms other than nuclear occupancy, such as translation, processing of nuclear message, receptor recycling, participate in the control of an end product's concentration.<sup>16</sup>

In this study, a difference was observed between the biochemical events of estrogen receptor modulation and the cellular morphologic condition. Biochemically no difference was noted between 2.5 mg and 5 mg of progestin at the 0.625 mg dose of estrogen. However, little progestational change was observed with the 2.5 mg dose whereas a clear progestational effect was associated with the 5 mg dose. There was no difference in the reduction of estrogen receptor levels between the 5 mg and 10 mg progestin doses in any of the estrogen treatment groups, but the 10 mg dose consistently resulted in a greater morphologic progestational response than did 5 mg of the progestin. More importantly, there was a homogeneous progestational effect in the endometrial tissues receiving 10 mg of progestin in contrast to the more focal changes resulting from 5 mg of the progestin.

In conclusion, conjugated equine estrogen therapy results in a dose-related increase in cytosolic estrogen receptor concentration. The biochemical events of progestin-induced antagonism of estrogen-stimulated increases in nuclear and cytoplasmic estrogen receptor concentration can be recorded at lower progestin doses than those inducing clear, homogeneous morphologic change. At the estrogen/progestin doses tested by sequential administration, 10 mg of medroxyprogester-

one acetate was required to invoke a homogeneous, secretory change in the endometrium.

Clinically it is difficult to base a plan for therapy on the data of only 13 patients. However, the results demonstrating a nonparallelism between the biochemical and morphologic events with progestin administration in postmenopausal endometrium make it imperative that treatment plans based on short-term biochemical findings, that is, receptor data, be evaluated very critically. It may be that maximal biochemical suppression of estrogen-stimulated events is sufficient to prevent subsequent neoplastic change (or that performing the biopsies in this study 2 or more days later would have demonstrated biochemical and morphologic parity). However, until long-term follow-up of biochemical data is available, morphologic criteria must remain the standard by which the antineoplastic postmenopausal hormonal replacement therapy is judged.

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# Heat flux and oxygen consumption of the pregnant uterus

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Heat flux (conductive and convective heat) and oxygen consumption of the pregnant uterus and its content were measured simultaneously in the same group of pregnant ewes during the acute postoperative period, during a chronic resting period, and during  $\alpha$ - and  $\beta$ -adrenergic-receptor stimulation with norepinephrine and ritodrine. Results indicated four conclusions. First, an excellent correlation existed between heat flux and oxygen consumption in the acute and chronic resting condition as well as during increasing uteroplacental vascular resistance and decreasing blood flow produced by norepinephrine infusion; the correlation was not as good during ritodrine infusion. Second, during rest, about 85% of heat generated by the pregnant uterus is eliminated through the uteroplacental circulation while the remaining heat diffuses through the myometrium. Third, during decreasing uteroplacental blood flow and elevated resistance, the pregnant uterus is able to maintain a normal thermostasis by widening the temperature difference in the blood entering and leaving the uterus and by increasing the myometrial heat exchange; oxygen consumption also is maintained at normal level through increase in oxygen extraction. Fourth, with the exception of uteroplacental circulation, the circulatory, metabolic, and thermal conditions of the pregnant ewe are not different after 5 hours from 5 to 7 days after the surgical procedure. (AM J OBSTET GYNECOL 1986;154:462-70.)

**Key words:** Metabolism, blood flow, adrenergic stimulation

It has been known since the pioneering work of LaVoisier almost two centuries ago that heat is constantly generated as the result of oxygen metabolism by the living tissues. Systemic and regional oxygen consumption has been measured in humans and in other animal species by many investigators, and the results have been compared to heat production under different experimental conditions. The heat generated during oxidative metabolism usually dissipates by different pathways according to the organ or region under study.

The pregnant uterus and its contents (fetus and umbilicoplacental unit) represent anatomically and physiologically a very complex system composed of various living components with differing metabolic activities. Throughout fetal growth and development, fetal metabolic activities increase progressively, and therefore heat production also increases. This heat must either dissipate through the surrounding structures (amniotic fluid, uterine walls) or pass from the fetal blood to the

maternal blood across the placental circulation. Temperature measurements have consistently shown that the fetal temperature is higher than the maternal even during induced hyperthermia or hypothermia.<sup>1-4</sup>

Many authors have measured oxygen consumption of the fetus alone or of the entire pregnant uterus and its content<sup>5, 6</sup> (for reviews, see References 7 and 8). However, quantitative assessment of the instantaneous relationship between oxygen consumption and heat production as well as of the pathways of heat dissipation in the resting state and during altered circulatory conditions have not been made.

The present studies were designed to provide answers to the following questions: (1) What is the relationship between oxygen consumption and heat production of the pregnant uterus and its content in sheep in the resting state? (2) What are the pathways for heat loss from the contents of the pregnant uterus, and what is the quantitative contribution of each route? (3) How do the heat production and the oxygen consumption of the pregnant uterus and its content respond to changes in the uteroplacental circulation produced by adrenergic receptor stimulation?

In addition, this series of experiments provided us the opportunity to examine the cardiovascular, metabolic, and thermal status of the same pregnant sheep in the acute (5 hours) and chronic (5 to 7 days) periods following the surgical procedure. We thought that this information would be important to shed light on the

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controversy surrounding acute versus chronic animal experimentation.

### Material and methods

Ten healthy ewes with singleton pregnancy (97 to 125 days' gestation) were used for these studies. The surgical procedures were performed under aseptic techniques with use of intermittent intravenous doses of pentobarbital and diazepam supplemented by local infiltration anesthesia (1% Xylocaine). The techniques for implanting catheters in the descending aorta, inferior vena cava and main uterine vein have been described previously<sup>9,10</sup>; these catheters served to monitor pressures and heart rate and to collect blood samples anaerobically.

To monitor the total uterine blood flow, the internal iliac artery was exposed at the site of its exit from the descending aorta; all of the branches supplying non-uterine structures were ligated. The vessel was then fitted with an electromagnetic flow transducer of appropriate size. The flowmeter used in these studies was a square wave type (Dienco Company, Los Angeles, California) that has been used in our laboratories for the last 5 years. Each flow transducer was calibrated in vitro by methods we have used for many years, which have been described in detail elsewhere.<sup>11,12</sup> The error of our method of measuring flow with this new type of flowmeter does not exceed  $\pm 5\%$ .

In addition, the following special technical features were used in these experiments.

**Definition and measurements of heat flux.** We defined heat flux as the sum of conductive heat transferred across the uterine surface and convective heat transferred via the uterine circulation.

**Temperature measurements.** To monitor conductive heat loss from the uterine surface, we used a thermopile transducer made into a thin plate measuring 3 to 4 cm in diameter (Thermometrics Corp., San Diego, California, HA-18/-18-15P). This plate was implanted under the serosal surface and in direct contact with the myometrium of the pregnant horn. The transducer cables were exteriorized through a stab wound in the back of the animal.

We tested the temperature recorded with this plate in different areas of the pregnant horn and found that the difference from one area to another does not exceed 2%; therefore we selected the site of implantation at the anterior aspect of the pregnant uterine horn.

Monitoring of the heat loss through the uterine circulation was accomplished by introducing into the descending aorta and the main uterine vein of the pregnant horn (through one of its branches) thermistor tip catheters (Yellow Springs Instrument Co., Yellow

Springs, Ohio, YSI 520). The cables of both thermistors were exteriorized through the back of the animal.

**Calibration of the thermistors and thermopiles.** The thermistors were calibrated in a well-stirred water bath with use of a standard and differential thermometer sensitive to 0.01° C of temperature change. The resistance changes of the thermistor were read on a potentiometer, and the signal was fed into a dynographic recorder.

The thermopile plate used to monitor the uterine surface temperature was calibrated by the manufacturers to provide a thermal constant expressed as watts per square meter per millivolt. In this thermopile the relationship between its voltage output and heat flow is a straight line. We checked the calibration factor of the five transducers used in our laboratory by the method of Nuckols and Piantaolosi.<sup>13</sup> Since the error we found was less than 5%, we decided to use the manufacturer's calibrations in our dynamographic recording, which was set to give 0.1 mV/cm.

### Calculations of heat flux

**Convective heat loss.** Convective heat loss through the uterine circulation was calculated with use of equation 1 derived from the Fick principle:

$$CVH = c \cdot \dot{Q}U (V-A) \quad (1)$$

where CVH = convective heat loss,  $\dot{Q}U$  = total uterine blood flow, V-A = the temperature difference (in degrees Centigrade) in the uterine venous and arterial blood, and c = specific heat content of blood (0.87 calorie/ml/degree C). With this formula, the total heat transferred through the uterine circulation can be expressed as calorie per minute or as a function of the weight of the uterus and its content as described below.

**Conductive heat loss.** We calculated the total conductive heat loss across the pregnant uterus with use of equation 2 as follows:

$$CDH = K \cdot S \cdot \frac{K_1 \times 1000}{60} \quad (2)$$

where CDH = conductive heat loss, K = thermal constant of the thermopile transducer (watts per square meter/mv), S = surface area of the pregnant uterus (square meter),  $K_1$  = conversion factor of 0.8606 which when multiplied by 1000% converts the unit of watts into units of calories per minute.<sup>2</sup> This equation provides values for heat exchange across the entire pregnant uterus, which may be expressed as calories per minute or as a function of the pregnant uterine weight.

**Calculation of the surface area of the uterus.** The surface area of the pregnant uterus (S in equation 2) was calculated as follows:

First, on exposing the pregnant uterus, two dimen-



sional measurements were obtained with use of a paper tape.

Second, we assumed that the pregnant uterus has the shape of a prolate spheroid, which is formed by the rotation of an ellipse about its major axis. By measuring the longitudinal and horizontal axis ( $a$  and  $b$ ), the surface area ( $S$ ) can be calculated with use of equations 3 and 4 as follows<sup>14</sup>:

$$S = 2\pi \left[ b^2 + \frac{ab}{e}(\sin^{-1} e) \right] \quad (3)$$

$$e = \frac{\sqrt{a^2 - b^2}}{a} \quad (4)$$

where  $S$  = surface area,  $a$  and  $b$  are the two measured axes of the pregnant uterus, and  $e$  = eccentricity of the spheroid.

**Oxygen consumption.** Oxygen consumption of the pregnant uterus and its contents was computed from the product of the blood flow and arteriovenous oxygen content differences with use of equation 5 derived from the Fick principle:

$$\dot{V}O_2 = \dot{Q}U (A-V) O_2 \quad (5)$$

Where  $\dot{V}O_2$  = oxygen consumption,  $\dot{Q}U$  = total uterine blood flow, and  $AO_2$  and  $VO_2$  = the oxygen content of the arterial and venous blood of the uterine circulation simultaneously measured with the flow.

Arterial pressure, heart rate, mean and phasic uterine blood flow (electromagnetic method), blood  $PO_2$ ,  $PCO_2$ , pH, oxygen saturation and contents, and hemoglobin were measured by techniques previously described.<sup>9, 10</sup>

**Experimental protocols.** The following three experimental protocols were carried out on this series of pregnant animals.

*Comparison of the acute and chronic postoperative states.* Data on the circulatory, metabolic, and thermal conditions of the same animal were obtained in the acute period following the operation and 5 to 7 days later.

In the collection of the acute data, each animal was allowed 5 hours of recovery from the operation. By that time the animal was awake and standing or moving in its cage, which was placed in an air-conditioned room maintained at 22° C.

The following parameters were recorded continuously for 45 to 60 minutes: (1) maternal heart rate, (2) phasic and mean arterial pressure, (3) phasic and mean total uterine blood flow, (4) arterial and uterine venous temperatures, and (5) conductive heat exchange at the myometrial surface. Arterial and uterine venous blood samples were collected every 10 minutes for determinations of blood respiratory gases, pH, and hemoglobin concentrations. The average readings of the mean uterine blood flow recorded during each one of these 10-

minute periods was used to calculate oxygen consumption and heat flux for that period.

For collection of the data on the chronic state we allowed the animal a period of 5 to 7 days of recovery from the operation. The animal received daily injections of antibiotics and was allowed free access to food and water. On the study day the ewe was brought to the same room at the same time of the day of the acute experiment. The same parameters listed above were again recorded for 45 to 60 minutes.

*Correlation between heat flux and oxygen consumption in the resting state.* This part of the experimental protocol was aimed at providing information on the relationship between heat production and oxygen consumption of the pregnant uterus and its content between 97 and 135 days of gestation. The data obtained from each animal during the acute period (5 hours) and those collected during the control period of 5 to 7 days later were taken as the resting values for that animal on that day. Both convective and conductive heat losses recorded simultaneously with oxygen consumption were grouped for the various 10-minute periods.

*Behavior of heat flux and oxygen consumption during  $\alpha$ - and  $\beta$ -adrenergic stimulation.* For those studies which were conducted in the chronic period<sup>1</sup> the same 10 pregnant ewes were used. Following collection of the control values on arterial pressure, heart rate, total uterine blood flow, aortic, uterine venous, and myometrial temperatures and blood gases, norepinephrine (1.25  $\mu$ g/kg/min) or ritodrine (7  $\mu$ g/kg/min) was administered by a constant intravenous infusion for a period of 30 minutes. This was followed by a recovery period during which the drug infusion was discontinued.

During both the periods of adrenergic-receptor stimulation and recovery, circulatory and temperature parameters were recorded continuously while arterial and uterine venous blood samples were collected twice during each period.

Only the effects of one given adrenergic agent were studied on any one day; an interval of 1 to 2 days was allowed before subjecting the animal to another test. The same animal was used for several tests of  $\alpha$ - and  $\beta$ -adrenergic-receptor stimulation for the duration of the pregnancy.

Following the termination of the studies, we performed a total hysterectomy and weighed and measured the pregnant uterus and its content and then individually the fetus and the placenta. In this way we were able to express the heat flux and oxygen consumption values as a function of the weight of the pregnant uterus and its content.

**Statistical analysis.** In analyzing the data for the acute and chronic states and responses to  $\alpha$ - and  $\beta$ -adrenergic-receptor stimulation, each animal served as its control. The mean, standard deviation, and standard

error were calculated, and analyses of variance for repeated measurements were done. For calculation of statistical significance, the Scheffe test was used, and  $p$  values of  $<0.05$  were considered significant. To compare oxygen consumption and heat flux, linear regression analysis was used by means of the IBM mainframe University of California (Los Angeles) computer facilities.

## Results

**Comparison of the status of the animal in the acute and chronic postoperative periods.** The animals tolerated the anesthesia and surgical procedures very well and were apparently in a steady-state condition within 3 to 4 postoperative hours.

Table I lists the values for the various circulatory, metabolic, and thermal parameters obtained at 5 hours and 5 to 7 days after surgery.

The values for arterial pressure and heart rate during the two periods were not significantly different. Uterine blood flow was significantly lower ( $p < 0.01$ ) 5 hours after the operation than after 5 to 7 days. Arterial  $PO_2$ ,  $PCO_2$ , and pH were not significantly different during the two periods. Arterial and uterine venous oxygen contents were lower ( $p < 0.05$ ) during the chronic than the acute period, but the arterial/venous difference was closely similar. Oxygen consumption of the pregnant uterus averaged about 19 ml/min at 5 hours after the operation and increased to about 21 ml/min 5 to 7 days later; heat flux increased from 98 to 111 calorie/min; both differences were not significant.

During the acute postoperative period, approximately 85% of the heat generated by the pregnant uterus and its content was dissipated by convection through the uterine circulation while the remaining was exchanged across the uterine surface by conduction. During the chronic period the amount of heat lost through the uterine circulation increased markedly, probably because of the increase in uterine blood flow; during the same period, however, the amount of heat lost through the uterine surface significantly decreased.

**Relationship between heat flux and oxygen consumption.** Fig. 1 shows the data for the relationship between heat flux (sum of conductive and convective heat loss) and oxygen consumption during the resting state (acute and chronic periods) and during  $\alpha$ - and  $\beta$ -adrenergic-receptor stimulations. Also presented are the data for the relationship between the differences in oxygen content and temperature of the blood entering and leaving the uterus.

An excellent correlation ( $r = 0.9746$ ) existed between the two factors that reflect the metabolic activities of the pregnant uterus and its content, whether during the acute or the chronic resting condition (Fig. 1,A). A similar degree of correlation between oxygen con-

**Table I.** Data comparing the cardiovascular, metabolic, and thermal status of the animals in the acute (5 hours) and the chronic (5 to 7 days) periods after the operation (values are mean  $\pm$  1 SE)

	Acute	Chronic
Maternal heart rate (bpm)	124 $\pm$ 6	115 $\pm$ 6
Mean arterial pressure (mm Hg)	99 $\pm$ 3	94 $\pm$ 3
Uterine blood flow (ml/min)	618 $\pm$ 60	802 $\pm$ 82*
Arterial $PO_2$ (mm Hg)	90.2 $\pm$ 2.7	86.9 $\pm$ 2.2
Arterial $PCO_2$ (mm Hg)	31.5 $\pm$ 1.5	39.0 $\pm$ 1.8
Arterial pH	7.549 $\pm$ 0.03	7.508 $\pm$ 0.01
Arterial oxygen saturation (%)	93.2 $\pm$ 0.9	91.9 $\pm$ 0.8
Arterial oxygen content (ml/100 ml)	13.5 $\pm$ 0.6	11.3 $\pm$ 0.7†
Uterine venous oxygen content (ml/100 ml)	10.6 $\pm$ 0.6	8.6 $\pm$ 0.6†
Oxygen content difference (ml/100 ml)	2.90 $\pm$ 0.2	2.61 $\pm$ 0.2
Uterine oxygen consumption (ml/min)	18.7 $\pm$ 2.7	20.9 $\pm$ 2.1
Total uterine heat flux (calorie/min)	98.0 $\pm$ 13.4	111.5 $\pm$ 9.5
Convective heat loss (calorie/min)	86.2 $\pm$ 12.9	107.3 $\pm$ 8.3
Conductive heat loss (calorie/min)	12.0 $\pm$ 3.9	4.2 $\pm$ 1.9†
Arterial temperature ( $^{\circ}$ C)	39.09 $\pm$ 0.11	39.70 $\pm$ 0.12
Uterine venous temperature ( $^{\circ}$ C)	39.26 $\pm$ 0.08	39.86 $\pm$ 0.12

\* $p < 0.01$ .

† $p < 0.05$ .

sumption and heat flux existed during  $\alpha$ -adrenergic-receptor stimulation with norepinephrine (Fig. 1,B). In the case of the data obtained during  $\beta$ -adrenergic-receptor stimulation the correlation was not as good (Fig. 1,C); this is probably due to complex metabolic activities of  $\beta$ -agonists. Of course, the presence of uterine blood flow on both axes contributed a great deal to this correlation. When the blood flow was removed from the figure and the arterial/venous oxygen content and temperature differences of the blood entering and leaving the uterus at rest were plotted (Fig. 1,D), the correlation between these two parameters remained highly significant. From these data we were able to calculate the caloric equivalent of each unit of oxygen consumed, which was 1 ml of oxygen = 5.2 calories.\* This value is very close to the theoretical figure of 4.9 Kcal/L of oxygen, which could be obtained assuming that the pregnant uterus and content burn primarily glucose and amino acids.

\*In a preliminary abstract (San Francisco, California: Society for Gynecologic Investigation, thirty-first annual meeting, March 21-24, 1984) we reported a caloric equivalent of 7.42 Kcal/L; this value was based on a few experiments and was in error.



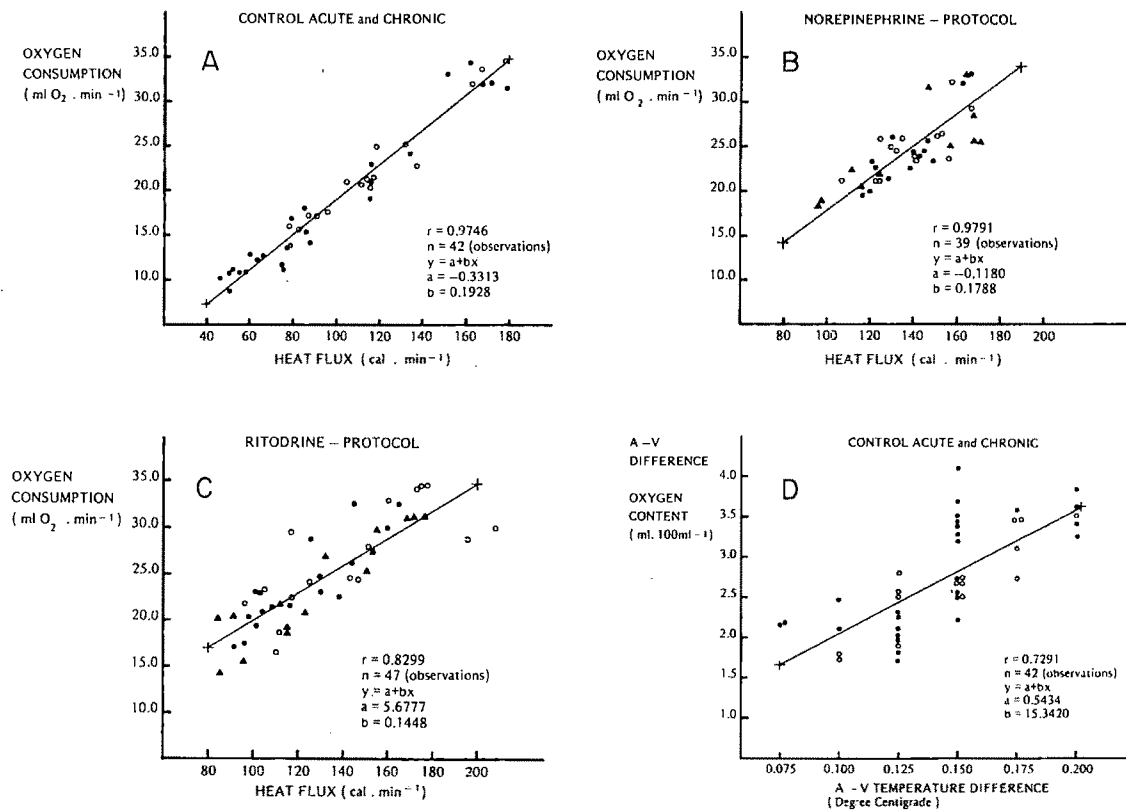


Fig. 1. A to C show the relationship between heat flux and oxygen consumption during the resting state and during  $\alpha$ - and  $\beta$ -adrenergic receptor stimulation. D shows the relationship between the differences in oxygen content and temperature of the blood entering and leaving the uterus.

### Effects of adrenergic stimulation

**$\alpha$ -Receptors.** Fig. 2 and Table II present the data on the effects of  $\alpha$ -adrenergic-receptor stimulation with norepinephrine on the circulatory, metabolic, and thermal functions in the pregnant sheep. Heart rate and uterine blood flow decreased significantly during norepinephrine infusion, whereas arterial pressure and uterine vascular resistance increased. All of these parameters returned to near control values during recovery. This pattern of cardiovascular response to  $\alpha$ -adrenergic stimulation was similar to those previously reported from our laboratories.<sup>10</sup>

Despite the marked decrease in uterine blood flow, heat flux and oxygen consumption did not change significantly during  $\alpha$ -adrenergic-receptor stimulation (Fig. 2 and Table II). This was due to a marked increase in the temperature and oxygen content differences of the blood entering and leaving the uterus.

Although the total heat flux during  $\alpha$ -adrenergic stimulation remained within control ranges, the distribution of heat loss changed considerably. As Table II shows, the loss of heat through the uterine circulation (convective heat loss) decreased while the amount of heat lost through the myometrial surface more than doubled during norepinephrine infusion.

**$\beta$ -Receptors.** Fig. 3 and Table II present the data on

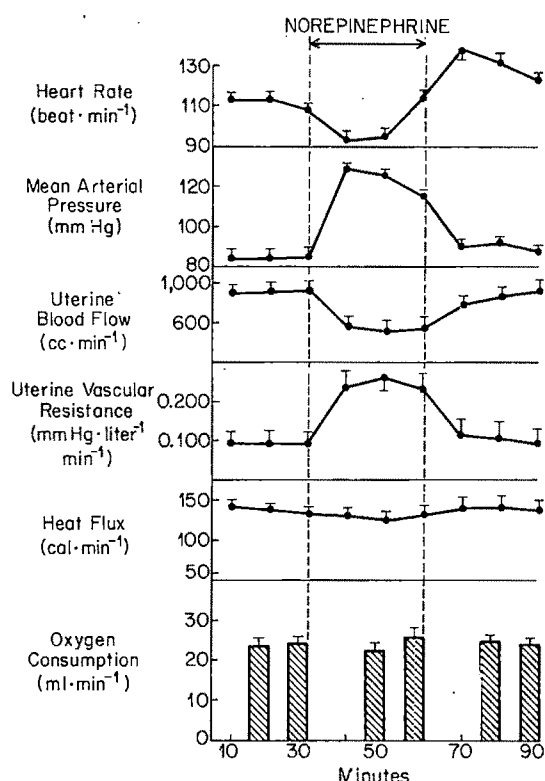
the circulatory, metabolic, and thermal responses to  $\beta$ -adrenergic-receptor stimulation with ritodrine. Heart rate increased markedly during the infusion and remained elevated during the recovery period. Mean systemic arterial pressure and total uterine blood flow decreased by an average of 10%; uterine vascular resistance did not change significantly. These responses were similar to those previously reported.<sup>10</sup>

Uterine oxygen consumption, total uterine heat flux, and the patterns of heat exchange distribution between conductive and convective loss did not change significantly during ritodrine infusion (Table II).

### Comment

The data derived from these series of experiments provide information on (1) the overall condition of the pregnant animal in the acute and chronic postoperative states, (2) the total heat production of the pregnant uterus and its content including the quantitative assessment of the pathways of heat dissipation and the relationship to oxygen consumption, and (3) the impact of the changes in uteroplacental hemodynamics on uterine metabolic activities. It would be appropriate, however, to begin the discussion with a critique of the methods.

**Methods and assumptions.** Since the present series



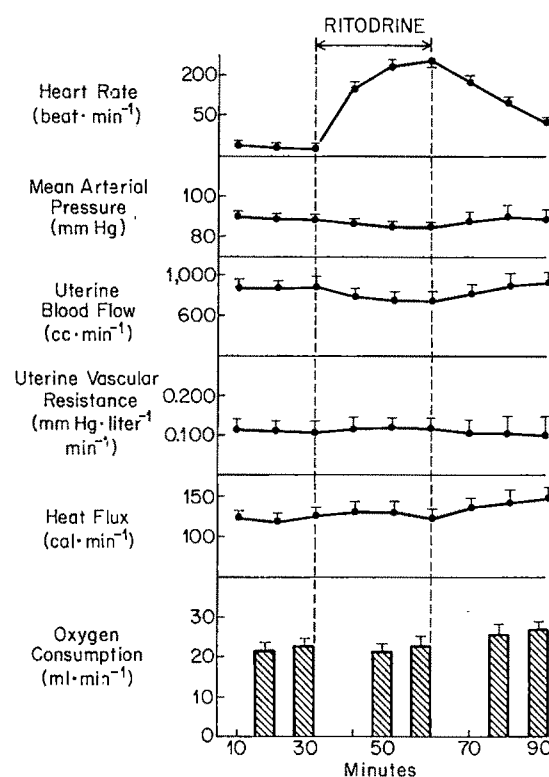
**Fig. 2.** Response of heart rate, arterial pressure, uterine blood flow, uterine vascular resistance, heat flux, and oxygen consumption to norepinephrine (values are mean  $\pm$  1 SE).

of experiments involved measurements of different parameters as well as the use of various assumptions, a discussion of their validity and the possible sources of errors is warranted.

**Flow measurements.** The blood flow to the pregnant uterus was monitored by the electromagnetic method that has been used in our laboratories for over 25 years. Although the electronic design of flowmeters has changed considerably during this period of time, the basic principles underlying this method as well as the calibration techniques of the transducers have remained as described in various publications.<sup>11, 12</sup>

In the present series of experiments we used the most recently developed flowmeter, which has excellent baseline stability, and the error on repeated measurements in acute and chronic experimental conditions does not exceed 5%. In our opinion, and in our hands, this method is preferable to the diffusion-equilibration technique with use of antipyrine in which the error on repeated measurements is greater.

**Measurements of oxygen contents and temperatures.** Oxygen content was measured on intermittently drawn blood samples with use of blood  $PO_2$ , saturation, and hemoglobin. We realize that this method is less accurate than one using modern apparatus which gives oxygen content directly. We believe, however, that this error is probably insignificant because the same technique was



**Fig. 3.** Response of heart rate, arterial pressure, uterine blood flow, uterine vascular resistance, heat flux, and oxygen consumption to ritodrine (values are mean  $\pm$  1 SE).

used in all of the experiments, whether in acute or chronic state or during changing blood flow.

The temperature of the uterine surface was monitored from one area of the pregnant uterine horn. Preliminary acute measurements showed that the temperature of different areas did not vary by more than 2%. Of course, it is possible that in the chronic state or during changes in uterine blood flow, the temperature in different areas of the myometrium may vary. Here again the influence of this factor may not be great because the same techniques of implantation and measurements were used in all of the experiments; hence any error may be randomly distributed.

The same can be stated regarding the temperature of the uterine venous blood which was monitored from one uterine vein. It is possible that, during a given period and certain experimental conditions, one uterine vein may have a slightly different temperature than the other. We have no way of assessing the significance of this factor.

**Assumptions.** In estimating the surface area of the uterus, we assumed that its shape is that of a prolate spheroid. Of course, the shape of the uterus may be somewhat different from a purely designed geometrical spheroid. However, in the face of performing acute and chronic experiments in which direct measurements of the surface area can not be made, we

**Table II.** Data on the effects of  $\alpha$ - and  $\beta$ -adrenergic receptor stimulation with norepinephrine and ritodrine (values represent average  $\pm$  SE of 10 animals)

	$\dot{Q}U$	$(V-A)T$	$(A-V)O_2$	$\dot{V}O_2$	Total heat flux	Convection heat	Conduction heat
Control	206 $\pm$ 21	0.162 $\pm$ 0.01	2.82 $\pm$ 0.19	5.60 $\pm$ 0.33	31.2 $\pm$ 2.3	29.2 $\pm$ 2.4	1.9 $\pm$ 0.4
Norepinephrine	134 $\pm$ 17*	0.244 $\pm$ 0.02*	4.54 $\pm$ 0.45*	5.50 $\pm$ 0.39	31.0 $\pm$ 3.6	26.3 $\pm$ 3.5†	4.7 $\pm$ 0.6*
Recovery	204 $\pm$ 21	0.170 $\pm$ 0.01	2.85 $\pm$ 0.18	5.71 $\pm$ 0.43	31.2 $\pm$ 2.3	29.0 $\pm$ 2.5	2.1 $\pm$ 0.4
Control	203 $\pm$ 27	0.155 $\pm$ 0.01	2.75 $\pm$ 0.17	5.29 $\pm$ 0.31	27.1 $\pm$ 2.0	25.6 $\pm$ 2.2	1.4 $\pm$ 0.58
Ritodrine	181 $\pm$ 15†	0.175 $\pm$ 0.01†	3.04 $\pm$ 0.22†	5.23 $\pm$ 0.32	27.9 $\pm$ 1.6	26.2 $\pm$ 1.5	1.7 $\pm$ 0.53
Recovery	210 $\pm$ 13	0.170 $\pm$ 0.01	2.81 $\pm$ 0.12	5.94 $\pm$ 0.26	31.6 $\pm$ 3.6	30.3 $\pm$ 3.6	1.5 $\pm$ 0.52

$\dot{Q}U$  = Uterine blood flow in milliliters per minute per kilogram of pregnant uterus;  $(V-A)T$  = temperature difference (degree C) of the uterine venous and arterial blood;  $(A-V)O_2$  = oxygen content difference (ml/100 ml) of the arterial and uterine venous blood;  $\dot{V}O_2$  = total oxygen consumption in milliliters per minute per kilogram of uterine weight; total heat flux and conductive and convective heat loss are in calories per minute per kilogram of uterine weight.

\* $p < 0.001$ .

† $p < 0.05$ .

believe our assumption is valid, particularly since it was used in all of the experiments.

**Acute versus chronic experimentation.** A great deal of discussion has been carried out among investigators during the past 15 years about the appropriate time after an operation to perform experiments on animals (particularly pregnant sheep) which would not be influenced by anesthesia and surgical procedure. The times reported in the literature for the definition of "chronic" experiments vary from 24 to 36 hours to 5 to 8 days after surgery. Nearly all of these reports, however, base their time selection more on convenience than on scientific basis. In 1974 Assali et al.<sup>9</sup> reported data in which they compared the cardiovascular status of the ewe and its fetal lamb in acute experiments carried out under spinal or pentobarbital anesthesia to a series of chronic experiments. Their paper, however, dealt with retrospective studies carried out on different series of animals used for different experimental protocols during different years. Nevertheless, these authors concluded that certain types of surgical procedures and anesthesia may affect the experimental results more than others.

One group of researchers studied fetal metabolism during a period of 2 hours after the operation and repeated it 2 and 4 days later; the surgical procedure was performed under spinal and pentobarbital anesthesia in sheep. They observed no differences in umbilical blood flow (measured by diffusion-equilibration technique) and oxygen consumption values observed during these three periods of study. There was, however, some difference in fetal blood glucose and aminonitrogen between the acute and chronic states. The significance of these latter findings, however, is not clear.

The present data obtained from the same group of animals studied by the same experimental techniques

including the type of anesthesia show that the general cardiovascular, metabolic, and thermal functions of the ewe were not different after 5 hours from those observed 5 to 7 days after the operation. There was, however, one exception; the uterine blood flow was significantly lower during the acute than during the chronic postoperative state. Two facts may have contributed to this difference. The first, which is probably minor, is related to the continuous growth of the pregnant uterus and its content during the period that elapsed between the acute and chronic studies. The second factor, which is more important, is related to manipulation of the pregnant uterus during surgery to expose and instrument the blood vessels. These procedures almost invariably cause spasm of the uterine arteries and veins and an increase in myometrial tension, leading to an enhanced intramural resistance. All of these variables may contribute to an increase in uterine vascular resistance and reduced uterine blood flow. Based on these observations, we believe that any investigator who contemplates performing experiments involving the pregnant uterus and its vascular supply should not do them in the acute postoperative period.

**Heat production and oxygen consumption in the resting state.** The information available in the literature regarding heat production and dissipation of the pregnant uterus and its content is meager. Most of the studies dealt with temperature differences between the fetal and maternal blood and other structures. In one study thermocouples were implanted in the ewe and its fetus and their temperatures were compared for several days. Their conclusions were that the fetal brain was 0.4 to 0.8° C warmer than maternal aortic blood and that the fetus continuously loses heat to the mother.

Hart and Faber<sup>1</sup> measured temperature differences between fetus and mother in the rabbit and, with use of various assumptions, concluded that no more than

about one third of the fetal heat production is lost via the umbilical circulation.

More recently, Power and co-workers<sup>4</sup> investigated the fetal heat production and the fetomaternal heat movements in the pregnant sheep by injecting ice-cold saline solution into the amniotic fluid before and after interruption of the umbilical circulation. They placed thermistors in the maternal aorta, amniotic fluid, and fetal aorta. They did not monitor the heat loss through the myometrial surface. By this technique they estimated that about 84% of the total fetal heat production passes through the placenta.

Oxygen consumption of the pregnant uterus, on the other hand, has been measured by numerous investigators (for review, see References 7 and 8). The reported values vary according to (1) methodology including the technique of measuring blood flow and the experimental condition (acute or chronic), (2) weight definition (per unit of fetal weight or of the pregnant uterus and its content), and (3) the gestational age. In acute experiments carried out in sheep and goats with use of the antipyrine diffusion-equilibration technique, oxygen consumption values of the pregnant uterus and its content have varied from 4 to ~10 ml/min/kg.<sup>6, 15-17</sup> In previous studies using the electromagnetic method,<sup>18</sup> oxygen consumption values averaged 4.5 ml/min/kg of fetal weight. The values observed in the present series of experiments, which averaged 6.1 ml/min/kg, are consistent with those of others in which identical methods were used.

The results obtained from this series of experiments in which direct and indirect calorimetric measuring techniques were applied to the pregnant uterus and its contents provide a new insight into the problem of heat production and pathways of heat dissipation at rest and during circulatory alterations.

The data obtained in the resting state, whether a few hours or some days after surgery, show that the production of heat by the pregnant uterus and its content bears a very close relationship with the amount of oxygen consumed. They further show that under the resting condition, the largest portion of this heat (85%) is lost through the uterine circulation, leaving only a small fraction to diffuse across the myometrial surface.

These results agree with the estimates of Power et al.<sup>4</sup> but are at variance with those obtained from pregnant rabbits by Hart and Faber<sup>1</sup> who estimated that no more than about one third of the fetal heat production is lost via the umbilical circulation. These workers, however, based their estimate on the temperature difference between the fetal and maternal blood and used different species of animals.

The results of our experiments further show (1) that both the heat flux and the oxygen consumption of the

pregnant uterus increased during the time that elapsed between the acute and chronic studies and (2) that the ratio of heat loss between convective and conductive pathway changed. The increase in both oxygen consumption and heat flux is most likely related to the increase in uterine blood flow that occurred during the chronic period. The increase in flow could also explain the changes in the magnitude of heat lost by convection and by conduction. For, as will be shown below, when uteroplacental vasoconstriction is present, more heat is lost through the myometrium and less through the uterine circulation.

**Effects of adrenergic stimulation.** The systemic and uterine effects observed in this series of experiments during  $\alpha$ - and  $\beta$ -adrenergic-receptor stimulation were not different from those reported previously.<sup>10</sup> A rise in the arterial pressure and uterine vascular resistance occurred simultaneously with the decrease in uterine blood flow during norepinephrine infusion. Minor changes in pressure, flow, and resistance occurred during  $\beta$ -stimulation.

But despite the marked fall in blood flow produced by norepinephrine, neither the oxygen consumption nor the total heat flux changed significantly. This was caused by widening of the difference in oxygen content and the temperature difference of the blood entering and leaving the uterus.

The ability of the pregnant uterus to compensate for the decrease in blood flow by extracting more oxygen has been observed by various investigators. However, this is the first series of observations that show that this ability extends to heat production. The data further show that, despite the maintenance of a normal heat flux during uteroplacental vasoconstriction produced by norepinephrine, the distribution of heat loss through the two main pathways changed. With the rise in uterine vascular resistance and decreased flow, the quantity of heat lost through the uterine circulation decreased while that through the myometrium increased. These findings indicate that the fetoplacental system is able to sustain marked changes in the uteroplacental circulation without impairing its ability to eliminate the heat produced by tissue metabolism. This ability rests on (1) widening the temperature difference between the uterine venous and arterial blood and (2) increasing the heat dissipation across the myometrial surface.

This hypothesis receives support from the observations made with  $\beta$ -adrenergic-receptor stimulation during which the uteroplacental vascular resistance did not change significantly. Under these circumstances the total heat flux as well as its two components, the conductive and convective heat losses, did not change significantly.



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## Glucose threshold for macrosomia in pregnancy complicated by diabetes

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We analyzed 205 diabetic women treated with insulin during pregnancy to assess the effects of several maternal factors on the development of fetal macrosomia. A total of 95 women were selected for study because they had clearly defined gestational criteria, two or more daytime glucose profiles during the third trimester, and no other complications known to affect fetal growth. The incidence of macrosomia was not found to increase significantly until the mean glucose concentration reached 130 mg/dl; macrosomia occurred in 65% of mothers with glucose values  $\geq 130$  mg/dl compared with 27% in those with lower values. Other factors strongly associated with fetal macrosomia were maternal weight and insulin dosage. Multiple logistic analysis was performed to control for each risk factor and to obtain estimates of the relative risk for macrosomia. The risk of macrosomia was two times greater in women with mean glucose concentrations  $\geq 130$  mg/dl, approximately threefold in women whose weight exceeded 80 kg, and one and one half times greater in women with insulin dosages more than 80 units/day. We conclude that several maternal factors in addition to glucose concentration play important roles in the development of fetal macrosomia among diabetic women and that the glucose concentration threshold for macrosomia may exceed 130 mg/dl. (*AM J OBSTET GYNECOL* 1986;154:470-5.)

**Key words:** Glucose concentrations, fetal macrosomia, diabetes, hyperglycemia

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The incidence of macrosomia in pregnancies complicated by diabetes is more than twice that for non-diabetic women.<sup>1</sup> For many years, investigators have sought to understand the factors that contribute to the development of macrosomia in infants of diabetic mothers. In 1933, Skipper<sup>2</sup> first hypothesized that excess adipose tissue in infants of diabetic mothers was a consequence of maternal hyperglycemia. In addition, he suggested that these infants might be hyperinsulinemic. In 1954, Pedersen<sup>3</sup> proposed that accelerated fetal growth in pregnancies complicated by diabetes occurred as a result of fetal hyperglycemia leading to elevated levels of fetal insulin which acted as a growth promoting factor.

Fetal growth appears to be affected by many factors in addition to maternal glucose levels. In nondiabetic women, maternal factors that have been shown to be related to macrosomia include parity, maternal size, weight gain, and prolonged pregnancy. Other determinants of birth weight that have been linked to excessive fetal size in nondiabetic women include fetal sex, history of a large infant, and race.<sup>4</sup> The influence of these factors on fetal size in diabetic women treated with insulin during pregnancy has not been previously investigated.

In this retrospective investigation of pregnant diabetic women who required insulin for control of hyperglycemia, we examined the relationship between maternal glucose levels and other maternal factors that may potentially contribute to macrosomia.

### Material and methods

The hospital records of all pregnant diabetic women treated with insulin and who were delivered of their infants at Parkland Memorial Hospital between July, 1974, and July, 1984, were reviewed. A total of 205 women were identified and 110 were excluded because of: (1) known chronic hypertension documented by elevated blood pressures in the first 20 weeks of pregnancy, antihypertensive therapy antedating the pregnancy, or a history of related complications (for instance, cerebrovascular accident or chronic renal insufficiency); (2) advanced vascular complications of diabetes, including women with diabetes classified as F or R according to White; (3) delivery of malformed infants; (4) gestational criteria not meeting the requirements of the study as defined below; (5) twin gestations; (6) an insufficient number of glucose measurements as defined below; and (7) preeclampsia early in the third trimester. Women with mild preeclampsia managed conservatively near term were not excluded.

The remaining 95 women included in the study were grouped according to White's classification,<sup>15</sup> with those women who had fasting hyperglycemia diagnosed only after pregnancy was confirmed identified as gestational diabetics.<sup>5</sup>

**Management.** Diabetic women were hospitalized in the Parkland high-risk pregnancy unit at the time of initial presentation. Management methods for these women have been described previously.<sup>6</sup> Briefly, single morning doses of NPH insulin or split morning and evening doses of regular and NPH insulin were used with the goals of achieving fasting glucose concentrations <115 mg/dl and preprandial glucose levels <150 mg/dl. All women were counseled regarding a 30 to 35 kcal/kg/day diet, according to the American Diabetes Association recommendations.<sup>7</sup> Serial sonography was begun at the time of initial presentation in known diabetic women and at the time of diagnosis in women with gestational diabetes. Sonography was repeated at 3- to 4-week intervals. Additional hospital admissions were arranged on an individual basis for insulin adjustments or antenatal complications. All patients were admitted to the hospital between 32 and 36 weeks' gestation and remained there until delivery. Delivery was effected typically by 38 weeks' gestation, after confirmation of fetal lung maturity.

**Mean glucose level.** A mean glucose value was calculated for each woman from a profile of glucose determinations performed during the final hospitalization. Glucose samples were drawn at 7:00 AM after an overnight fast and preprandially at 11:00 AM, 4:00 PM, and 9:00 PM. At least two such profiles were required for women to be included in this study. The great majority of samples were obtained by venipuncture and assayed for plasma glucose by the oxidase method with the use of an AutoAnalyzer. A few, more recent glucose determinations were obtained from finger capillary samples with the use of an Ames glucometer.

**Macrosomia.** Fetal macrosomia was defined as fetal weight greater than or equal to the ninetieth percentile adjusted for gestational age, in accordance with birth weight standards reported by Brenner et al.<sup>8</sup>

**Gestational criteria.** Gestational age was calculated from the first day of the woman's last menstrual period. This was corroborated either by fundal height at detection of fetal heart tones<sup>9</sup> or by sonographic determination of gestational age before 26 weeks when the latter agreed within 2 weeks with fetal age as predicted by the last menses. When the last menses was unknown or not considered reliable because of a history of oligomenorrhea or oral contraceptive use, gestational age was determined according to fundal height at the onset of fetal heart tones and sonography before 26 weeks' gestation or a minimum of two consistent sonographic examinations before 26 weeks' gestation. Women presenting for prenatal care after 26 weeks' gestation and those whose gestational estimates did not meet the above criteria were excluded.

**Statistical analysis.** Cross tabulations between each anticipated maternal risk factor and macrosomia were

**Table I.** Selected demographic characteristics of 95 insulin-treated diabetic women and their infants

Characteristic	n	%
Diabetes classification		
Gestational	36	37.9
A	1	1.1
B	19	20.0
C	23	24.2
D	16	16.8
Maternal age (yr)		
<20	19	20.0
20-24	28	29.5
25-29	19	20.0
30-34	16	16.8
≥35	13	13.7
Parity		
0	40	42.9
1	25	26.6
2	17	18.1
≥3	12	12.7
Race		
Black	46	49.5
White	29	31.2
Hispanic	18	19.4
Fetal sex		
Male	44	51.8
Female	41	48.2
Birth weight percentiles		
<10	3	3.2
10-25	9	9.5
25-50	10	10.5
50-75	20	21.1
75-90	8	8.4
≥90	45	47.4

evaluated individually, with the use of  $\chi^2$  analysis. Those factors identified as important were then entered in a multiple logistic regression equation, so as to estimate the independent effect of each factor on the likelihood of macrosomia, with the other factors controlled. An estimate of the odds ratio (approximate relative risk) for each factor was obtained from the logistic coefficients.<sup>10</sup> Finally, the probability of macrosomia, given various combinations of risk factors, was calculated from the logistic model.

## Results

The distributions of women and their infants according to selected demographic characteristics are shown in Table I. Almost 40% of the women had gestational diabetes and the remainder were approximately evenly divided between White Classes B, C, and D. Two thirds of the women were less than 25 years old, and 14% were 35 years of age or older. Approximately 40% were primigravid. The racial distribution of the diabetic women reported here was similar to that of the general Parkland Hospital obstetric population. Specifically, approximately 50% were black, 30% white, and 20% Hispanic.

Also shown in Table I are distributions by fetal sex and birth weight percentiles. Almost half of the infants exceeded the ninetieth percentile for birth weight. Twenty-two (22%) of the infants weighed >4 kg. The mean birth weight was  $3465 \pm 784$  gm; the smallest and largest infants weighed 1600 and 5950 gm, respectively. The mean gestational age at delivery was 37 weeks and 94% of the infants were delivered at ≤38 weeks.

**Macrosomia and maternal factors.** With macrosomia defined as a birth weight equal to or exceeding the ninetieth percentile for gestational age, statistically significant associations between macrosomia and selected maternal characteristics are shown in Table II. Class of diabetes, maternal age, and race were not found to have significant associations with macrosomia. Maternal weight gain during pregnancy was not studied because reliable prepregnancy weights were not available.

Macrosomia was significantly more common among women of higher parity. The incidence was 66% in women of parity ≥2 as compared with 40% in women of lower parity. Similarly, diabetic women previously delivered of a large infant (≥4000 gm) frequently gave birth to an infant greater than the ninetieth percentile in the present pregnancy. Specifically, of the 54 multigravid women with a prior large infant, 79% again had a macrosomic infant. In contrast, only 48% of those without prior large infants were delivered of macrosomic infants.

Maternal weight at admission during the final hospitalization was also significantly associated with macrosomia. Women who weighed <70 kg had a 16% incidence of macrosomia, whereas those who weighed between 70 and 80 kg were twice as likely to give birth to macrosomic infants. The incidence of macrosomia increased even further in women who weighed ≥80 kg.

**Glucose threshold for macrosomia.** Subjects were divided into eight groups according to the mean daytime glucose concentration during the final hospitalization. These mean concentrations were calculated from a total of 431 daytime glucose profiles available for the 95 women studied. The mean number of daytime glucose profiles was 4.5 and the range was two to 18 profiles. Shown in Table III is the incidence of macrosomia at each maternal glucose level. The incidence of macrosomia did not consistently increase until a mean glucose concentration of 130 mg/dl. When maternal glucose concentrations <130 mg/dl are compared with those levels exceeding this threshold, the incidence of macrosomia increased from 27% to 65%, respectively.

A consistent linear relation between maximum insulin dose and macrosomia was not demonstrated. However, women whose maximum insulin dose exceeded 80 units/day had a significantly higher incidence of macrosomia than those whose maximum insulin dose

**Table II.** Incidence of macrosomia according to selected maternal characteristics

Characteristic	Macrosomia*				$\chi^2$
	No		Yes		
	n	%	n	%	
Parity					9.90†
0	25	62	15	37	
1	14	56	11	44	
2	7	41	10	59	
≥3	3	25	9	75	
History of infant >4000 gm					4.06†
Yes	3	21	11	79	
No	21	52	19	48	
Maternal weight (kg)					16.8†
<70	16	84	3	16	
70-79	12	67	6	33	
80-89	8	47	9	53	
90-99	2	23	10	77	
100-109	6	50	6	50	
≥90	5	31	11	69	

\*Birth weight equal to or exceeding the ninetieth percentile for gestational age.

†p < 0.05.

**Table III.** Incidence of macrosomia in relation to mean maternal glucose concentration during final hospitalization

Mean glucose level (mg/dl)	Total No. of infants	Macrosomic infants		$\chi^2$
		n	%	
80-89	5	1	20	19.45*
90-99	7	4	57	
100-109	9	1	11	
110-119	14	5	36	
120-129	9	1	11	
130-139	17	10	59	
140-149	10	7	70	
>150	24	16	67	
Total	95	45	47	

\*p < 0.05.

was less than 80 units/day (65% versus 39%, respectively; p = 0.02).

**Multiple logistic analysis.** In order to estimate the independent effect of each of the maternal factors found to be significantly associated with macrosomia in the univariate analysis, a multiple logistic analysis was performed (Table IV). In equation 1, all factors found to be important in the univariate analysis were entered into a model in which the dependent variable was macrosomia (1 = yes; 0 = no). When other factors were controlled for, parity no longer had a significant effect on the likelihood of macrosomia. Therefore, the model was reestimated after parity was excluded. The results (equation 2) show little change in the estimated logistic coefficients for the other maternal factors.

From examination of the odds ratios in Table IV, it can be seen that in comparison with mothers weighing <70 kg, those weighing between 70 and 80 kg were about twice as likely to have a macrosomic infant. Sim-

ilarly, those weighing >80 kg were approximately three times as likely to have a macrosomic infant. When mean glucose level and the other factors were controlled, mothers whose maximum insulin dose exceeded 80 units/day were one and one half times as likely to have a macrosomic infant compared with those with lower insulin doses. Finally, mothers whose mean glucose levels were ≥130 mg/dl were about twice as likely to have a macrosomic infant compared with those with lower mean glucose levels.

To examine the relation between one risk factor and macrosomia for varying levels of another risk factor, interaction effects between risk factors were tested by means of the logistic model. No significant interaction effect was found.

From the estimated coefficients of each variable in equation 2, an estimated logistic score can be calculated for any given patient with a specific combination of risk factors. To simplify interpretation, the intercept 0.02



**Table IV.** Estimated coefficients for multiple logistic analysis of maternal factors influencing the incidence of macrosomia

Variable	Equation 1			Equation 2		
	Coefficient	$\chi^2$	Odds ratio*	Coefficient	$\chi^2$	Odds ratio*
Intercept	-0.11	0.91	—	0.02	0.003	—
Parity $\geq 1$	0.28	0.90	1.31 (0.87-1.75)	—	—	—
Maternal weight						
>70 and $\leq 80$ kg	0.52	1.45	1.68 (1.09-2.58)	0.56	1.67	1.75 (1.14-2.72)
>80 kg	1.23	10.10†	3.42 (2.32-5.05)	1.31	11.54‡	3.69 (2.51-5.42)
Maximum insulin dose >80 units/day	0.52	3.71†	1.68 (1.28-2.20)	0.48	3.42‡	1.6 (1.25-2.12)
Mean glucose $\geq 130$ mg/dl	0.79	8.43†	2.20 (1.68-2.89)	0.84	9.88†	2.32 (1.77-3.04)

\*Approximately equal to relative risk.<sup>10</sup> Confidence limits corresponding to 1 SE are shown in parentheses.

†p < 0.05.

‡p < 0.06.

can be subtracted from the total score, so that a patient without any identifiable risk factors would have a score of 0. The resulting logistic scores can then be transformed into estimates of the probability for macrosomia (Fig. 1). For example, a woman with an insulin dose <80 units/day, a mean glucose concentration <130 mg/dl, and a weight <70 kg has a 50% probability of having a macrosomic infant. A woman with an insulin dose exceeding 80 units/day, a mean glucose level >130 mg/dl, and weighing >80 kg has a 93% risk.

### Comment

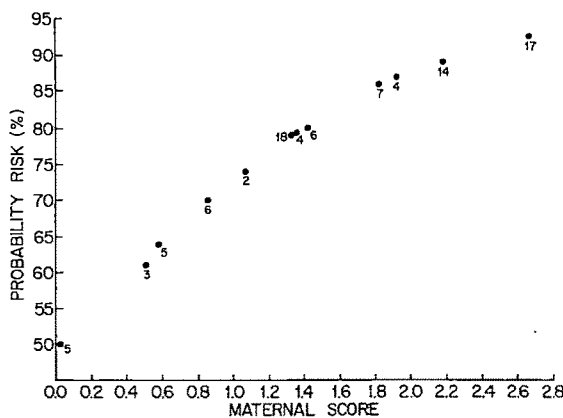
It has long been recognized that fetal macrosomia is a complication of diabetes mellitus.<sup>2</sup> According to the Pedersen hypothesis,<sup>3</sup> maternal hyperglycemia due to diabetes leads to fetal hyperinsulinemia that results in excessive growth. Indirect evidence in support of this hypothesis comes from both animal and human studies. Specifically, a relationship between fetal hyperinsulinemia and increased subcutaneous fat has been reported in rhesus monkeys and rats.<sup>11-13</sup> In humans fetal pancreatic cell volume has been shown to be proportional to maternal glucose concentrations.<sup>14</sup> Similarly, Sosenko et al.<sup>15</sup> have correlated increased umbilical cord insulin C-peptide levels with macrosomia.

Although maternal hyperglycemia is clearly a factor implicated in the development of macrosomia, the evidence is often conflicting. Several investigators<sup>16-18</sup> have reported an association between maternal glucose control and macrosomia while others<sup>19-21</sup> have not been able to substantiate this finding. For example, Pedersen,<sup>3</sup> when proposing his hypothesis in 1954, observed that women whose diabetes had been well controlled were delivered of smaller infants than those with less stringent metabolic control. Later investigators such as Karlsson and Kjellmer,<sup>22</sup> in their classic report linking maternal glucose control to perinatal outcome, were

unable to confirm a relationship between maternal glucose concentrations and macrosomia. Recently, Knight et al.<sup>23</sup> concluded that factors other than maternal glucose concentration must play roles in the development of macrosomia. These conflicting results and observations such as those by Knight et al. suggest that many factors influence the incidence of macrosomia in pregnancies complicated by diabetes.

In this investigation, macrosomia was evaluated in relation to several maternal characteristics in order to identify those factors and their interactions that are associated with excessive fetal size. The exclusion of women with factors known to impair fetal growth (for instance, vascular complications) and selection of strict criteria for calculation of gestational age were intended to permit more accurate investigation of those factors associated with macrosomia. The results indicate that macrosomia among diabetic women is multifactorial in origin. Not only is glucose concentration a determinant of macrosomia, but maternal characteristics such as weight and a history of large infants also play important roles. When these factors were controlled for each others' influences by means of multiple logistic analysis, a multifactorial etiology of fetal macrosomia was further substantiated. Women with several concomitant risk factors, for example, insulin dose exceeding 80 units/day, mean glucose exceeding 130 mg/dl, and weight >80 kg, had an extremely high (93%) risk of fetal macrosomia.

The results of this investigation also clearly substantiate a relationship between maternal glucose concentration and excessive fetal growth. However, the relationship was not linear and the incidence of macrosomia was not consistently increased until the mean glucose concentration reached  $\geq 130$  mg/dl. It should be emphasized that there was a great deal of variation in the incidence of macrosomia for glucose values <130



**Fig. 1.** Relationship between estimated maternal risk score, derived from logistic analysis, and the probability of macrosomia. The number of women represented by each point is shown.

mg/dl, much of which was likely due to small sample size. Importantly, additional studies are needed to address the impact of euglycemia on the frequency of macrosomia since few diabetic women reported on here had glucose concentrations in the 80 to 85 mg/dl range described for women with normal pregnancies.<sup>24</sup>

It might be viewed as surprising that insulin dose had an independent effect on fetal macrosomia even when maternal glucose control was controlled for. As insulin does not cross the placenta, the importance of greater insulin requirements may be that it reflects a state of greater insulin resistance and greater abnormalities in fatty acid and lipid metabolism, which are also factors reported to affect fetal growth.<sup>14, 25</sup> Alternatively, this insulin dosage link to macrosomia may only reflect maternal obesity, type II diabetes, and associated insulin insensitivity.

In conclusion, we are convinced that fetal macrosomia in diabetes is multifactorial in origin and is not singularly the consequence of maternal hyperglycemia. Specifically, we found that maternal obesity, maternal insulin requirement, maternal glucose level, and history of a large infant were associated with fetal macrosomia. Thus factors known to be associated with excessive fetal size in nondiabetic women must also be considered when women with well-controlled diabetes inexplicably give birth to macrosomic infants. Finally, and pending further studies, the maternal glucose concentration threshold for macrosomia in diabetic women may exceed euglycemic levels.

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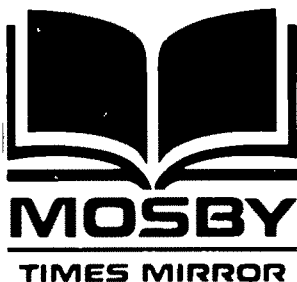
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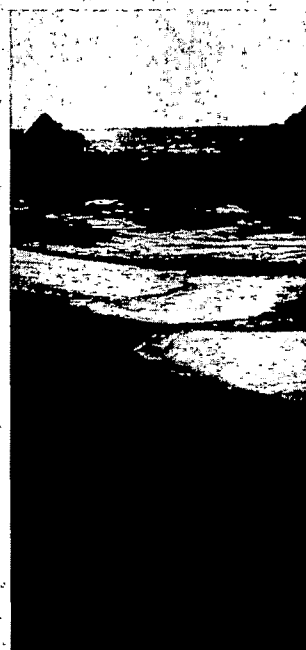
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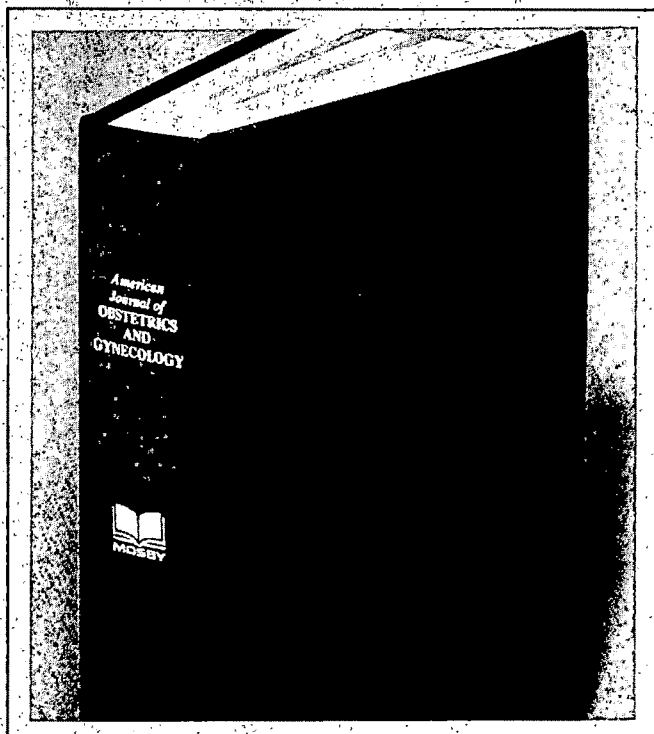
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## Warnings

**Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.**

The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks:

1. **Thromboembolic Disorders and Other Vascular Problems**—An increased risk of thromboembolic and thrombotic disease associated with the use of OCs is well established. Three principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OCs are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.

**CEREBROVASCULAR DISORDERS**—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

**MYOCARDIAL INFARCTION**—An increased risk of myocardial infarction associated with the use of OCs has been reported, confirming a previously suspected association. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preclinical toxemia) the higher the risk of developing myocardial infarction, regardless of whether the patient was an OC user or not. OCs, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be taken into consideration. The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; it is quite likely that the same synergistic action exists, but perhaps to a lesser extent.

**RISK OF DISEASE**—In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism, including coronary thrombosis, is directly related to the dose of estrogen used in OCs. Preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the United States.

**ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES**—A large prospective study carried out in the U.K. estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OCs according to age, smoking habits and duration of use. The overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), the risk being concentrated in older women in those with a long duration of use and in cigarette smokers. It was not possible, however, to examine the interrelationships of age, smoking and duration of use, nor to compare the effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. The available data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. The estimates of risk of death for each method include the combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OCs) plus the risk attributable to pregnancy or abortion in the event of method failure. This latter risk varies with the effectiveness of the contraceptive method. The study concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OCs in women over 40 who smoke. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with OCs increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes or history of preclinical toxemia and, especially by cigarette smoking. The physician and the patient should be alert to the earliest manifestations of thrombophlebitis (thrombophlebitis and thrombotic disorders, e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four- to six-fold increased risk of postoperative thromboembolic complications has been reported in oral contraceptive users. If feasible, OCs should be discontinued at least 4 weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization.

**PERSISTENCE OF RISK OF VASCULAR DISORDERS**—Findings from one study in Great Britain involving cerebrovascular disease and another study in the United States concerning myocardial infarction suggest that an increased risk of these conditions in users of OCs persists after discontinuation of the OC. In the British study, the risk of cerebrovascular disease remained elevated in former OC users for at least six years after discontinuation. In the U.S. study, an increased risk of myocardial infarction persisted for at least 9 years in women 40- to 49-years-old who had used OCs for five or more years. The findings in both these studies require confirmation since they are inconsistent with other published information.

2. **Ocular Lesions**—There have been reports of neuro-ocular lesions, such as optic neuritis or retinal thrombosis, associated with the use of OCs. Discontinue OC medication if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. **Carcinoma**—Long-term continuous use of progestins, either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina and liver. Certain synthetic progestogens, now commonly contained in OCs, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case-control studies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the cases found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time OCs were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs. Several studies have found no increase in breast cancer in women taking OCs or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with OCs, found an excess risk in the subgroups of OC users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of OCs has been well-documented. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with OCs. Close clinical surveillance of all women taking OCs is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use OCs.

4. **Hepatic Tumors**—Benign hepatic adenomas have been found to be associated with the use of OCs. One study showed that OC formulations with high hormonal potency were associated with a higher risk than lower potency formulations. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of OCs. Two studies relate risk with duration of use of OCs, the risk being much greater after 4 or more years of OC use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women taking OCs. The relationship of these drugs to this type of malignancy is not known at this time.

5. **Use or Immediately Preceding Pregnancy, Birth Defects in Offspring and Malignancy in Female Offspring**—The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence at the present time that OCs further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OCs. Furthermore, a high percentage of such exposed women (from 30 to 50%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including OCs, in pregnancy. One case-control study has estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. It is recommended that, for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed

period and further use of OCs should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient is apprised of the potential risks to the fetus, and the advisability of continuation of the pregnancy should be discussed in the presence of these risks. It is also recommended that women who discontinue OCs with the intent of becoming pregnant use an alternate method of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no information is available on which to base this recommendation. The administration of progestogen-only or progestogen-combination to induce withdrawal bleeding should not be used as a test of pregnancy.

6. **Gallbladder Disease**—Studies report an increased risk of surgically confirmed gallbladder disease in users of OCs and estrogen. One study, an increased risk appeared after 2 years of use and doubled after 4 or 5 years of use. In one of the other studies, increased risk was apparent between 6 and 12 months of use.

7. **Carbohydrate and Lipid Metabolic Effects**—A decrease in glucose tolerance has been observed in a significant percentage of patients on OCs. For this reason, prediabetic and diabetic patients should be carefully observed while receiving OCs. An increase in total phospholipids has been observed in patients receiving OCs. Three studies have been performed with Tri-Levlen Tablets (Levonorgestrel and Ethinyl Estradiol Tablets Triphasic Regimen) formulation and no significant alteration in metabolism were noted, with the exception of a slight increase in triglyceride levels in one study. The clinical significance of these findings remains to be defined.

8. **Elevated Blood Pressure**—An increase in blood pressure has been reported in patients receiving OCs. In some women, hypertension may occur within a few months of beginning OC use. In the first year of use, the prevalence of women with hypertension is low and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer use and in the fifth year of use is two- and a-half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop hypertension when given OCs. Hypertension that develops as a result of taking OCs usually returns to normal after discontinuing the drug.

9. **Headache**—The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, severe, requires discontinuation of OCs and evaluation of the cause.

10. **Bleeding Irregularities**—Breakthrough bleeding, spotting and amenorrhea are frequent reasons for patients discontinuing OCs. Breakthrough bleeding, as in all cases of irregular bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Chances of bleeding are higher with estrogen content, while potentially useful in minimizing menstrual irregularities, should be done only if the patient has a history of bleeding from the vagina. Women with a past history of oligomenorrhea or secondary amenorrhea or women who have irregular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuing OCs. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. **Ectopic Pregnancy**—Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. **Breast-feeding**—OCs given in the postpartum period may interfere with lactation. There may be a decrease in the quality of the breast milk. Furthermore, a small fraction of the hormonal agents in OCs has been identified in the milk of nursing mothers. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of OCs should be deferred until the infant has been weaned.

**Precautions—GENERAL**—1. A complete medical and family history should be taken prior to initiation of OCs. The pre- and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, OCs should not be prescribed for longer than 5 years without a physical examination and PAP smear being performed. 2. Under the influence of estrogen-progestogen preparations, uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed while taking OCs. If pathology has been excluded, time or a change to another formulation may solve the problem. 4. Patients should be advised that OCs may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving OC therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution. 7. OC users may have disturbances in normal triptophan metabolism which may result in a relative pyridoxine deficiency clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by OC therapy. Since the pregnant is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of OC therapy when relevant specimens are submitted. 10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OCs. Increased sulfobromophthalen retention. b. Increased prothrombin and factors VII, VIII, IX and X. decreased antithrombin III. c. Increased prothrombin-induced platelet aggregation. d. Increased thyroid-binding globulin (TBG) leading to increased circulating thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or T4 by radioimmunoassay. Free T4 resin uptake decreased reflecting the elevated TBG. Free T4 concentration is unaltered. e. Decreased preheparinized clotting time. f. Reduced in vitro metyrapone test.

**Information for the Patient**—See Patient Package Labeling.

**Drug Interactions**—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracyclines. **Carcinogenesis**—See "Warnings" section for information on the carcinogenic potential of OCs.

**Pregnancy**—Pregnancy Category X. See "Contraindications" and "Warnings."

**Nursing Mothers**—See "Warnings."

**Adverse Reactions**—An increased risk of the following serious adverse reactions has been associated with the use of OCs: thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hepatic gallbladder disease, benign hepatomas, congenital anomalies.

There is evidence of an association between the following conditions and the use of OCs, although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients receiving OCs and are believed to be drug-related: Nausea and vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first three months of use, as a general rule, are seen much less frequently or only occasionally; gastrointestinal symptoms (such as abdominal cramps and bloating), breakthrough bleeding, spotting, change in menstrual flow, dysmenorrhea, amenorrhea during or after treatment, temporary infertility after discontinuance of treatment, edema, chloasma or melasma which may persist; breast changes such as tenderness, enlargement and secretion, change in cervical erosion and cervical secretion, possible diminution in lactation when immediately postpartum, cholestatic jaundice, migraine, increase in size of uterine leiomyomata, rash (allergic), mental depression, reduced tolerance to carbohydrates, vaginal candidiasis, change in corneal curvature (steepening), intolerance to contact lenses. The following adverse reactions have been reported in users of OCs, and the association has been neither confirmed nor ruled out: premenstrual-like syndrome, cataracts, changes in libido, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, varicella, hemolytic uremic syndrome.

**Acute Overdose**—Serious ill effects have not been reported following acute ingestion of large doses of OCs by young or middle-aged women. Serious symptoms, such as nausea and vomiting, may occur in females.

**Dosage and Administration**—To achieve maximum contraceptive effectiveness, Tri-Levlen Tablets (Levonorgestrel and Ethinyl Estradiol Tablets—Triphasic Regimen) must be taken exactly as directed and at intervals not exceeding 24 hours. (If Tri-Levlen is started later than the first day of the first menstrual cycle of medication or postpartum, contraceptive reliance should not be placed on the first 7 consecutive days of administration. The possibility of ovulation and conception prior to initiation of medication should be considered.) Any time the patient misses 1 or 2 brown, white or light-yellow tablets, she should also use another method of contraception until she has taken a tablet daily for 7 consecutive days.

For full details on dosage and administration see prescribing information and package insert.

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 **BERLEX**  
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Incidence of discontinuations for medical reasons in long-term clinical studies was only 11.3%,<sup>2</sup> indicating remarkably high patient acceptance.

TRI·LEVLEN™ 21 TRI·LEVLEN™ 28



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See last page of advertisement for brief summary of prescribing information.



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# TRI-LEVLEN<sup>TM</sup>

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Second to none for low total hormonal dose in a combination OC

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With silver matte finish and slender profile, the case looks like a credit card holder or calculator. Women appreciate the neutral styling.

**Available in 21-day  
and 28-day dispensers**







Excellent cycle control

## Low overall incidence of breakthrough bleeding

In 77 U.S. and U.K. studies involving 3,546 women (35,036 total cycles), a low 5.7% overall incidence of breakthrough bleeding was reported.<sup>2</sup>

## Minimal impact on the following metabolic and hemodynamic parameters\*:

Lipids,<sup>3</sup> carbohydrate metabolism<sup>3</sup> and blood pressure<sup>2</sup> were not significantly affected in specific long-term studies.

# The confidence of 170 million cycles of experience worldwide

ious as well as minor side effects have been reported following the use of all oral contraceptives. These include thromboembolic disease. The physician should remain alert to the earliest manifestations of any symptom of serious disease and discontinue oral contraceptive therapy when appropriate. These data represent only the results of selected studies. Therefore, before prescribing, the physician should read and remain alert to data found in the complete prescribing information for the product. Please also see brief summary of prescribing information on the last page of advertisement.

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NEW FROM BERLEX

# TRI-LEVELLEN<sup>TM</sup>

Levonorgestrel and ethinyl estradiol tablets—Triphasic regimen

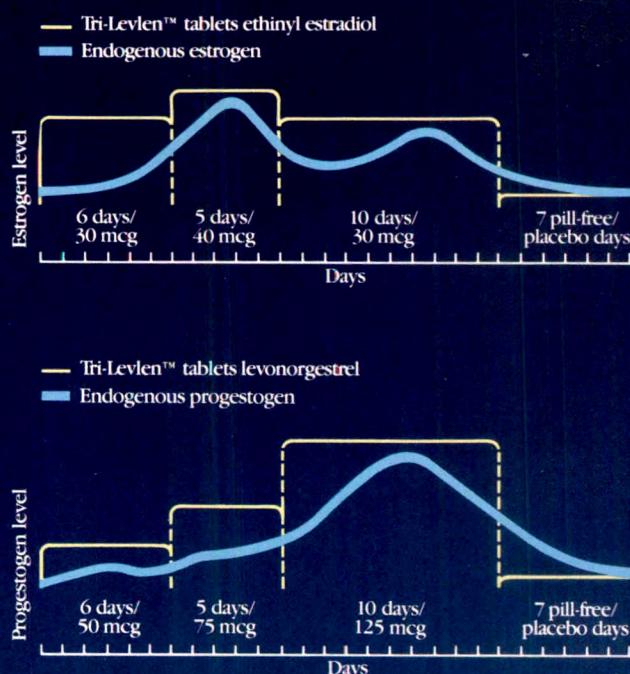
Second to none for low total hormonal dose in a combination OC

Follows a woman's natural hormone patterns

## Phased doses of both levonorgestrel and ethinyl estradiol

The Tri-Levlen<sup>TM</sup> tablets phased dosing permits a decrease in total steroid dose without compromising effectiveness. Follows a woman's normal cycle for a natural approach to cycle control.

"...for any given estrogen-progestin combination, it is advisable to use that product which contains the lowest doses of estrogen and progestin consistent with the needs of the patient."





NEW FROM BELL

The low-dose triphasic OC  
with the confidence that  
comes from experience



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**Table III.** Aerobic bacteria isolated from groups of 10 women using different methods of contraception

Aerobic bacteria	Barrier (n = 11)	Oral contraceptives (n = 27)	IUD (n = 29)
Gram-positive cocci			
Micrococci	—	1	—
Coagulase-negative staphylococci	—	4	1
Viridans streptococci	—	6	8
Microaerophilic streptococci	—	2	6
Group B streptococci	1	1	—
Enterococci	1	1	1
Gram-positive rods			
<i>Lactobacillus</i> sp.	9	6	7
Diphtheroids	—	2	3
Gram-negative rods			
<i>G. vaginalis</i>	—	4	3

inflammatory disease in IUD users over 25 years of age is remarkably less than in young women.<sup>2, 8</sup>

The present results confirm that it is a good policy to avoid IUD insertion in nulliparous women aged less than 25. However, sexual activity in general, as seen from the flora of the oral contraceptive users, shifts the cervical flora from a lactobacilli-dominated to an anaerobe-dominant direction. Another explanation might be that oral contraceptives affect the cervical environment and make it suitable for anaerobic bacteria. The vaginal bacterial content is slightly affected by the menstrual cycle.<sup>11</sup> The samples in the present study were taken during the luteal phase or in the premenstrum except in two of the 10 IUD users who had amenorrhea induced by the local effect of levonorgestrel.<sup>15</sup> In IUD users there was a connection between subjective complaints of vaginal discharge, the amine sniff test, cytologic findings, and the number of anaerobes found.<sup>9, 10</sup> Patients having typical nonspecific vaginitis can be treated without thorough bacteriologic studies.<sup>9</sup> A finding of *G. vaginalis* does not always mean nonspecific vaginitis, which may also be caused by anaerobes.<sup>10</sup> These facts were also noted in the present study. It can be questioned whether the increased risk of pelvic inflammatory disease in IUD users is connected with the increased number of anaerobes found in the cervix. The IUD users of the present study all had a levonorgestrel-releasing IUD, which has been shown to be safe in terms of a very low infection rate in a controlled clinical study.<sup>19</sup> The results showed that the cervical flora of these IUD users was rich although the infection rate was low. The levonorgestrel released locally makes the cervical mucus scanty but highly viscous so that it probably serves as a barrier for ascend-

**Table IV.** Anaerobic bacteria isolated from groups of 10 women using different methods of contraception

Anaerobic bacteria	Barrier (n = 5)	Oral contraceptives (n = 20)	IUD (n = 37)
Gram-positive cocci			
<i>Peptococcus asaccharolyticus</i>	2	1	6
<i>P. prevotii</i>	—	1	2
<i>P. magnus</i>	—	2	3
<i>Peptococcus</i> sp.	—	—	2
<i>Gaffkya anaerobia</i>	—	—	2
<i>Peptostreptococcus anaerobius</i>	—	1	1
<i>Peptostreptococcus</i> sp.	—	—	1
Gram-positive rods			
<i>Lactobacillus</i> sp.	1	6	1
<i>Bifidobacterium</i> sp.	—	3	2
<i>Actinomyces naeslundii</i>	—	—	1
<i>Actinomyces meyeri</i>	1	—	—
<i>Eubacterium</i> sp.	—	—	1
<i>Propionibacterium acnes</i>	—	1	2
Gram-negative rods			
<i>Bacteroides bivius</i>	—	2	4
<i>B. oralis</i>	—	2	4
<i>B. capillosus</i>	—	—	1
<i>B. vulgatus</i>	—	1	—
<i>B. ureolyticus</i>	—	—	1
<i>Bacteroides</i> sp.	1	—	3

ing infections. Infections caused by *Chlamydia trachomatis* or gonococci have been treated in patients using a levonorgestrel-releasing IUD. Thus levonorgestrel does not offer protection against sexually transmitted diseases.

In conclusion, the occurrence of anaerobic bacteria in the cervix in women using an IUD was significantly more common than in women using barrier contraception. Lactobacilli were predominant in those using a barrier method and only few anaerobes were found. A shift toward the dominance of anaerobes was also found in women using oral contraceptives. According to the results the cervical bacterial flora of sexually active, healthy women of reproductive age is rich in anaerobes, which can be regarded as a normal finding in these women. Barrier contraception with a condom protects against this anaerobic shift and maintains a lactobacilli-dominated flora in the cervix.

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**Table I.** Single and multiple isolates from the cervix in women using different methods of contraception

	Method of contraception		
	Barrier (n = 10)	Oral contraceptives (n = 10)	IUD (n = 10)
No. of single isolates	6*	0	1
No. of multiple isolates	4	10	9

\*Mainly lactobacilli.

spectively. None of the vaginal or cervical smears showed atypia or findings evident of disease. In general the smears of women using barrier contraception or oral contraceptives contained fewer leukocytes and bacteria than those of IUD patients. All cultures for *T. vaginalis* were negative. Culture for *C. albicans* was positive in three of the women with a levonorgestrel-releasing IUD whereas the cultures were negative in the other two groups. The potassium hydroxide odor test was positive in three of the patients having a levonorgestrel-releasing IUD. Two of these complained of an odorous vaginal discharge whereas no subjects in the other groups did so. Anaerobic bacteria were isolated from the cervix in these three women with clinical signs of nonspecific vaginitis. One also had a positive culture of *G. vaginalis*.

**Bacteriologic findings.** The numbers of women having single or multiple bacteria isolated from the cervix are shown in Table I. Six of the women using barrier contraception had only one isolate, which was *Lactobacillus* sp. In contrast, women using oral contraceptives or an intrauterine contraceptive device had multiple isolates, except for one IUD patient, from whom only aerobic lactobacilli were isolated.

Table II shows the total number of aerobes and anaerobes isolated from the three groups using different methods of contraception. Statistically significantly more anaerobes were isolated from those using oral contraceptives or an IUD when compared with those using a barrier method. The trend was the same for the number of aerobes isolated. The different aerobic and anaerobic bacteria isolated are listed in Tables III and IV. *Lactobacillus* sp. was the dominant isolate in those using barrier contraception. Only one woman had group B streptococci and another in this group had enterococci. Only five anaerobes were isolated from this patient group and these anaerobic isolates were all from only two women. The number of aerobic bacteria isolated from those using oral contraceptives or an IUD was roughly the same (27 and 29) and the different species of bacteria did not differ greatly in these two groups. As for the number of anaerobic bacteria isolated from the women using oral contraceptives

**Table II.** Number of different types of aerobes and anaerobes isolated from groups of 10 women using different methods of contraception

Bacteria	Barrier	Oral contraceptives	IUD
Aerobes	11*	27	29*
Anaerobes	5†‡	20†	37‡
Total no. of isolates	16	47	66

\*p &lt; 0.01.

†p &lt; 0.01.

‡p &lt; 0.01.

or an IUD (20 and 37), the difference was significant when compared to those of the barrier group (p < 0.01), and a rich flora of anaerobes was found in those having an IUD. The greatest difference was seen between the anaerobic flora of women using barrier contraception and that of those having an IUD, as shown in Table IV. These women were equally active sexually and of the same age. They were also parous.

*G. vaginalis* was isolated from three women having an IUD. The occurrence of *G. vaginalis* was associated with a rich flora of anaerobes in these women, whereas the samples were devoid of lactobacilli. The women participating in the present study were healthy and had no infections except for the three IUD users with signs of anaerobic vaginosis. Thus the bacteria isolated from the cervix in these women of reproductive age can be regarded as part of the normal flora of the cervix in sexually active women using contraception.

### Comment

The bacterial flora of the cervix was more abundant and rich in anaerobes in women using an IUD compared with that of those using barrier contraception. Anaerobic gram-negative rods and gram-positive cocci had replaced lactobacilli in the IUD group. The bacterial flora of women using barrier contraception was rich in lactobacilli and only few anaerobic bacteria were found. This flora can be regarded as the most normal among these three groups. It was interesting to find that oral contraceptive users also had increased amounts of anaerobes when compared with the barrier group. The oral contraceptive users were younger than those having an IUD, which may have slightly affected the results. However, the age distribution of the barrier contraception and the IUD groups was similar and all were parous. The age difference between the oral contraceptive and IUD groups reflects our policy of prescribing oral contraceptives to young nulliparous women whereas IUDs are favored more in parous women and older age groups. The relative risk of pelvic

with only one sexual partner, with only a minor increase in the relative risk of acquiring tubal infertility during IUD use. In contrast, development of tubal infertility<sup>5</sup> and an increased risk of pelvic inflammatory disease<sup>6</sup> have been significant with the old-fashioned IUDs such as the Dalkon Shield and the Lippes Loop. Gynecologic infections are of the ascending type and an IUD with its tail in the cervix is thought to play a role.<sup>7</sup> Oral contraceptives offer some protection against pelvic inflammatory disease.<sup>2,8</sup> Recently, nonspecific vaginitis, *Gardnerella vaginalis*, and vaginal anaerobes in the etiology of nonspecific vaginitis have received much attention in the literature.<sup>9,10</sup> Nonspecific vaginitis is common in IUD users.<sup>10</sup> The normal vaginal bacterial flora has been studied by many investigators,<sup>11,12</sup> whereas less is known about normal cervical bacteriology in women of reproductive age.<sup>13,14</sup> The same bacteria are found in the vagina and cervix,<sup>11</sup> although differences among individuals are common.

The purpose of the present study was to investigate the relationship between cervical aerobes and anaerobes, different methods of contraception, clinical findings and possible subjective complaints. Another aim was to study the normal cervical bacteriology of sexually active, healthy women of reproductive age using different methods of contraception. Because of the association between IUD use and infection it was expected that women using an IUD would be colonized by more potential pathogens and more anaerobes when compared with those of other women.

### Material and methods

**Patients.** Patients attending the family planning outpatient clinic of the State Maternity Hospital in Helsinki, Finland, participated in the study after giving their informed consent. Ten women of a mean age of 22 years (range 17 to 29) used low-dose combination oral contraceptives. They had been taking oral contraceptives for ½ to 3 years. Ten women had a levonorgestrel-releasing IUD manufactured by Leiras Pharmaceuticals, Turku, Finland,<sup>15</sup> which they had had in place for 1 to 6 years (mean 3.6). The mean age of the patients using the IUD was 34 years (range 26 to 42). All IUD patients were parous whereas only two of the 10 oral contraceptive users were parous. The third group of 10 women used barrier contraception (condom) but all had requested an IUD. The mean age of this group was 33 years (range 22 to 42) and all were parous. All patients were interviewed and had a stable sexual relationship with only one sexual partner. Their general health was good and the patients had no apparent gynecologic disease. Antimicrobial agents had not been taken during the preceding month. The visits usually took place during the luteal phase of the cycle, except for two subjects in the oral contraceptive group

and one subject in each of the other two groups. Two of the IUD users had amenorrhea caused by local suppression of the endometrium by levonorgestrel.<sup>15</sup> The day of last sexual intercourse was ascertained. At the visit a gynecologic examination was performed and all patients had normal findings without cervicitis or other disease.

**Bacteriologic and other samples.** The sample for bacteriologic culture of the cervix was taken first. To minimize vaginal contamination the ectocervix was gently wiped with a piece of sterile compress, soaked in sterile physiologic saline solution. Samples for bacterial cultures were taken with swabs deep in the cervical canal. The swabs were placed in modified Stuart's transport medium (Transpocult, Orion Diagnostica, Espoo, Finland) and sealed immediately in anaerobic bags (Anaerocult P, Merck, Darmstadt, West Germany). The bacteriologic samples were processed within 2 hours at the Anaerobe Reference Unit of the National Public Health Institute, Helsinki, Finland. A culture sample for *Candida albicans* (yeasts) and *Trichomonas vaginalis* as well as material for routine cytologic vaginal and cervical smears was taken from the vaginal fornix. In addition, a potassium hydroxide test for amine odor was performed.

**Methods of bacterial culture.** In the laboratory the specimens were transferred to an anaerobic glove box (Capco Systems 800, Sunnyvale, California), where the swabs were immersed in 1 ml of physiologic saline solution and mixed vigorously with a vortex mixer. Aliquots of 100 µl were plated onto various selective and nonselective media (5% sheep blood, supplemented Muller-Hinton with and without antibiotics, biphasic human blood, supplemented brucella, kanamycin-vancomycin-laked blood, bacteroides bile esculin, neomycin-vancomycin, phenylethyl alcohol, and cadmium-fluoride-acriflavin-tellurite agar media) for aerobic (including *Neisseria gonorrhoeae* and *G. vaginalis*) and anaerobic organisms. Plates were incubated at 35° C in various atmospheric conditions (in air, 5% carbon dioxide, a gas mixture of 80% nitrogen, 10% carbon dioxide, 10% hydrogen) from 2 to 6 days. Facultative organisms were identified according to the *Manual of Clinical Microbiology*,<sup>16</sup> and anaerobes were determined according to the manuals of the Virginia Polytechnic Institute<sup>17</sup> and Wadsworth Medical Center.<sup>18</sup>

Poisson's *t* test was used for statistical calculation.

### Results

**Clinical findings.** All women were healthy and normal according to clinical gynecologic examination. They were also sexually active, as the last coitus had taken place 1 to 7 days before the clinical visit in eight, six, and eight patients of the groups of 10 women using barrier contraception, oral contraceptives, or IUDs, re-

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## Bacterial flora of the cervix in women using different methods of contraception

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Bacteriologic culture samples were taken from the cervix in three groups of 10 healthy, sexually active women using barrier contraception, oral contraceptives, or a levonorgestrel-releasing intrauterine contraceptive device. Culture samples for *Candida albicans* and *Trichomonas vaginalis* were taken, a cytologic vaginal smear was obtained, and an amine sniff test was performed; these were in addition to a routine gynecologic examination. Multiple bacteria were isolated from the cervix in women using oral contraceptives or an intrauterine contraceptive device, whereas lactobacilli alone dominated the flora of women using barrier contraception. Significantly more anaerobic bacteria were isolated from the cervix in oral contraceptive and intrauterine contraceptive device users when compared with the barrier method users. Symptoms and findings evident of anaerobic vaginosis were associated with the occurrence of anaerobic bacteria in the cervix of three patients using the intrauterine contraceptive device. The results showed that the cervical bacterial flora in sexually active healthy women is rich in anaerobes that can be regarded as a normal finding in women using oral contraceptives or intrauterine contraceptive devices. Barrier contraception with a condom prevents this anaerobic shift and maintains a lactobacilli-dominated flora in the cervix. (*AM J OBSTET GYNECOL* 1986;154:520-4.)

**Key words:** Bacterial flora, cervix, contraception, intrauterine contraceptive device.

The intrauterine contraceptive device (IUD) is a popular method of contraception in parous women in Finland. The cumulative gross termination rate of copper IUD use because of infection during a period of 60

months is around three per 100 acceptors in Finland.<sup>1</sup> Although this is a small number it is well known that IUD use increases the relative risk of pelvic inflammatory disease.<sup>2,3</sup> However, the risk is much more pronounced in nulliparous women aged less than 25 than in parous women over this age.<sup>2</sup> For example, among 871 nulliparous British women fitted with a Cu-7, the life-table rates for pelvic inflammatory disease after 30 months of use were 14.2 for women aged 16 to 19 and 1.2 for women aged 30 to 49.<sup>4</sup>

Recently, a relationship between the use of an IUD and tubal infertility was reported.<sup>5</sup> The modern copper-releasing IUDs are fairly safe, especially in women

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Based on their theory, leukocyte transfusions have been proposed for cure of habitual abortion.<sup>9</sup>

The results of a recent controlled trial of therapy in recurrent spontaneous abortions show that the outcome of subsequent pregnancies is significantly altered by injections of paternal cells as compared to the outcome after injections of autologous cells.<sup>11</sup> Seventeen of 22 women (77%) who had received their respective husbands' cells gave birth to viable infants afterward, as compared with 10 of 27 women who were given their own cells (37%). It may, however, be questioned whether the patients studied constitute a representative population of habitually aborting women, since 104 of 209 couples studied were excluded from the trial. The causes for exclusion were negative Rh factor, presence of cytotoxic antibody against paternal lymphocytes, pregnancy when examined, and residence outside the United Kingdom. The presence or absence of lymphocytotoxic antibody has not been convincingly shown to bear relation to the outcome of pregnancy, and only between 20% and 50% of multiparous women have such antibody.<sup>12</sup>

The absence of blocking antibody in habitually aborting women seems to be linked to an increased frequency of HLA antigen sharing among spouses.<sup>1-3</sup> Women with habitual abortion are abnormally sensitive to suppressive influences in two-way mixed lymphocyte culture when exposed to lymphocytes from their own husbands or control subjects.<sup>3</sup> This might reflect an aberrant immune reactivity against cellular antigens. Accordingly, Beer et al.<sup>2</sup> have found that a significant proportion of habitually aborting women are hyporesponsive to paternal HLA antigens, including both cellular and humoral factors, but this does not consistently correlate with D locus antigen sharing.<sup>2</sup>

Pretransplantation transfusions of blood containing leukocytes significantly increase human renal allograft survival<sup>13</sup> and are shown to generate both blocking antibodies directed against T-cell antigens<sup>14</sup> and suppressor cells.<sup>15</sup>

Specific subpopulations of trophoblast express specific trophoblast antigens linked to the HLA locus antigens,<sup>1</sup> and these trophoblast antigens serologically cross react with antigens on human lymphocytes.<sup>8</sup> We have found that blood transfusions given to women who habitually abort induce the production of previously absent MLC blocking antibody.<sup>16</sup>

The blocking antibody normally demonstrable in sera of women during<sup>4</sup> and after<sup>5</sup> normal pregnancy is usually absent in habitual abortion.<sup>3, 6</sup> This does not prove beyond doubt, however, that the blocking antibody itself is the sole necessary prerequisite for a successful pregnancy. There are habitually aborting women in whom the sera display mixed lymphocyte culture blocking capacity not different from that found in a normal fertile population.<sup>2</sup> According to our cur-

rent results, the absence or occurrence of blocking serum antibody predicts whether therapeutic success may be gained by giving blood transfusions to habitually aborting women. The strong blocking antibody in some women with habitual abortion may be signs of autoimmune disease, such as systemic lupus erythematosus (found by us, manuscript in preparation). Accordingly, the women who have strong blocking antibody and abort may have a pathogenetic mechanism underlying the abortions that is different from that in the women who abort in the absence of blocking antibody, and therefore the appropriate therapy will be different. Autoimmune disease may be aggravated by blood transfusions. Therefore we sincerely recommend that blood transfusions not be used for therapy against habitual abortion without preceding investigation of the immune reactions of the woman.

Since blood transfusions are potentially hazardous, they should be given only after careful consideration to patients who can benefit from the transfusions. Our results clearly indicate that a selected population of habitually aborting women, that is, those without blocking antibody, benefits from transfusions of blood from third-party donors.

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**Table III.** Blocking antibody before transfusions and pregnancy outcome in 28 women with primary habitual abortion

<i>Blocking before transfusions*</i>	<i>n</i>	<i>Pregnancy outcome after transfusions</i>
Yes†	7	2 Abortions 1 Abortion and 1 child‡
No (?)§	7	1 Abortion‡
No	14	2 Extrauterine pregnancies‡ 9 Children 5 Pregnancies¶

\*Blocking antibody in mixed lymphocyte culture demonstrated in serum sample before transfusions.

†Blocking significant.

‡See text.

§Blocking weak, not significant.

||Blocking absent.

¶Pregnancies of 37, 26, 29, and 15 weeks, respectively.

again. When coagulation in activated partial thromboplastin time was subsequently investigated, four of five had a definite prolongation, and they had auto-antibodies as signs of autoimmune disease, such as systemic lupus erythematosus. One of them is now well into her sixth pregnancy (week 16) and is taking 0.25 gm of acetylsalicylic acid per day. So far the activated partial thromboplastin time is within the normal limits during pregnancy.

The fifth of the women aborting again, who had strong blocking antibody before the transfusions (Tables II and III), had had five early spontaneous abortions before the transfusion series. She had a normal activated partial thromboplastin time when not pregnant. Her sixth pregnancy ended in another abortion after the transfusions. At that time the placenta had multiple severe infarctions. She became pregnant once more and then took 0.25 gm of acetylsalicylic acid daily from conception on. During the twelfth week of pregnancy, the activated partial thromboplastin time was very prolonged, and she then had positive tests for antibodies against cardiolipin and deoxyribonucleic acid. From the sixteenth week of pregnancy on, she took prednisolone 30 mg daily. After 4 weeks of prednisolone therapy, when she was in the twentieth week of pregnancy, the activated partial thromboplastin time was within normal limits. Prednisolone medication was continuously 30 mg/day. In the twenty-fourth week of pregnancy the anti-DNA antibody had returned to normal, and the cardiolipin antibody was decreased, although still at a much elevated level. Weekly ultrasound examinations as a control have shown perfect continuous growth of the fetus. Blood pressure was acceptable until the thirty-third week of pregnancy, when she developed massive proteinuria, hypertension, and edema. She was considered preeclamptic and underwent a cesarean section. The baby was a girl weigh-

**Table IV.** Blocking antibody before transfusions and pregnancy outcome in 21 women with secondary habitual abortion

<i>Blocking before transfusions*</i>	<i>n</i>	<i>Pregnancy outcome after transfusions</i>
Yes†	4	1 Abortion 1 Abortion and 1 pregnancy of 16 wk‡
No (?)§	2	2 Children
No	15	6 Pregnancies¶

\*Blocking antibody in mixed lymphocyte culture demonstrated in serum sample before transfusions.

†Blocking significant.

‡See text.

§Blocking weak, not significant.

||Blocking absent.

¶Pregnancies of 37, 33, 28, 25, 21, and 13 weeks.

ing 1680 gm and in good condition. This patient apparently suffers from previously undiagnosed systemic lupus erythematosus. It is notable that 12 years ago her elder sister died of systemic lupus erythematosus at the age of 30.

Thirty-eight women who had weak or absent blocking antibody before transfusions (Table II) all developed significant blocking serum capacity after transfusions. Twenty-five have become pregnant. One has aborted again (see below), two have had an extrauterine pregnancy each (see below), 11 have been delivered of healthy infants, and 11 are well into their pregnancies (Tables III and IV).

One woman with primary habitual abortion had had four spontaneous abortions, and the serum displayed no blocking activity (Tables II and III). After transfusions, she had significant blocking antibody and was told to try to become pregnant. Not until 30 months later did she manage to become pregnant. She aborted again in gestational week 11 and at that time was found to have completely lost the blocking antibody.

One of the women with primary habitual abortion had earlier had three spontaneous abortions and two extrauterine pregnancies. After the transfusion series she experienced another extrauterine pregnancy.

Another woman with primary habitual abortion had had three spontaneous abortions. A hysterosalpingogram showed postinfection damage to the fallopian tubes, one being a hydrosalpinx and the other surrounded by adhesions. She received a series of transfusions and subsequently had an extrauterine pregnancy.

### Comment

The Faulk-McIntyre theory for the maintenance of pregnancy via immune reactions against trophoblast-leukocyte cross-reactive antigens<sup>8</sup> is as yet unproved.

**Table I.** Pregnancy histories of the women

	No. of women	No. of abortions	No. of pregnancies
Primary habitual abortion	28	4.7 ± 1.8	4.7 ± 1.8
Secondary habitual abortion	21	4.7 ± 1.7	5.8 ± 1.9
Total	49	4.7 ± 1.8	—

women were not pregnant when investigated or when receiving blood transfusions.

The autologous sera of the women were investigated for capacity to block the reactions in one-way mixed lymphocyte culture, in which the women's cells were exposed to mitomycin-treated cells from their own husbands or pools of lymphocytes from blood donors.

Determinations of the women's blood groups included ABO antigens, rhesus antigens, including subgroups (CcDEe), Kell, Fy<sup>a</sup>, and high-incidence antigens such as Vel, Lu<sup>b</sup>, and Yt<sup>a</sup>. Those who were Kell positive were also tested for k (Cellano).

**Blood donors and transfusions.** Blood donors were chosen separately for each woman according to blood groups in the above-mentioned blood group systems. The risk of transferring acquired immune deficiency syndrome (AIDS) is presently a threat each time a blood transfusion is given. Considering this fact, we preferably gave our patients with habitual abortion blood from female blood donors well known to the staff at the blood center. (From May, 1985, on all blood donors are being tested for antibodies against human T-lymphotropic virus type III, and only blood from those with negative tests is being used.) To minimize the risk of hepatitis, we used only donors who had previously given blood at least six times without adverse effects on the recipients, and donors with positive serum tests for HBs antigen or raised serum levels of liver enzymes were excluded. The risk of virus activation after transfusion was diminished by keeping the donor blood in the refrigerator for 3 days.

Plasma was separated, and the buffy coat with the packed erythrocytes remained. The transfusion blood was cross matched against the serum of the recipients and red blood cells. After a negative cross match the leukocyte-rich erythrocyte concentrate was transfused. Pulses, blood pressures, and body temperatures of the patients were controlled before, during, and after the transfusions.

The women received three transfusions at intervals of 4 to 8 weeks. They were not pregnant during this period. Before each transfusion, a blood sample was obtained from the woman, and serum was separated for storage in the freezer.

**Assay of blocking antibody.** The blocking capacity of sera was investigated in one-way mixed lymphocyte culture.<sup>3</sup> Lymphocytes were separated from heparinized venous blood via Ficoll-Isopaque gradient centrif-

**Table II.** Blocking antibody before transfusions in 49 women with habitual abortion and pregnancy outcome after a series of three transfusions

Blocking before transfusions*	n	Pregnancy outcome after transfusions
Yes†	11	3 Abortions 1 Abortion and 1 child‡ 1 Abortion and 1 pregnancy of 16 wk
No (?)§	9	1 Abortion‡
No	29	2 Extrauterine pregnancies‡ 11 Children 11 Pregnancies¶

\*Blocking antibody in mixed lymphocyte culture demonstrated in serum sample before transfusions.

†Blocking significant.

‡See text.

§Blocking weak, not significant.

||Blocking absent.

¶Pregnancies of 37, 37, 33, 28, 26, 25, 21, 20, 15, 14, and 13 weeks, respectively.

ugation, washed repeatedly, and then diluted in RPMI 1640 (Flow Laboratories, Irvine, Scotland) supplemented with antibiotics, glutamine, and serum. The final serum concentration in cell cultures was 10%.

Serum was obtained from the habitually aborting women at time of their first immunologic investigation, before each blood transfusion, and 2 months after the third transfusion, when reactivity in one-way mixed lymphocyte culture was tested once again. Serum was either used at once or stored at -20° C. All sera were heat-inactivated for 30 minutes at 56° C.

The inhibition exerted by the serum of a woman was determined by comparing the stimulation of her cells in her own serum with the response in AB serum.<sup>3</sup> The effect of autologous serum was calculated as the percentage of the response in AB serum (100 × counts per minute in own serum/counts per minute in AB serum).

The significance of blocking capacity was calculated according to the Mann-Whitney U test. Student's *t* test was used for evaluating the different effects on the responses in one-way mixed lymphocyte culture exerted by autologous serum obtained before and after the blood transfusions.

## Results

As can be seen in Table I, both of two groups of women, consisting of 28 women with primary and 21 with secondary habitual abortion, respectively, had experienced 4.7 abortions.

Of the 49 women, 11 had strong blocking antibody before the transfusion series (Table II). Five of them became pregnant after the transfusions, and all aborted

# Transfusions of leukocyte-rich erythrocyte concentrates: A successful treatment in selected cases of habitual abortion

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Forty-nine women who suffered recurrent spontaneous abortion of unknown etiology were studied for cellular reactivity and blocking antibody in one-way mixed lymphocyte culture before and after their receipt of three transfusions of leukocyte-rich erythrocyte concentrates from third-party donors. Those 38 of the 49 women who had no blocking antibody all developed significant blocking activity after the transfusion series. Twenty-five of them have become pregnant since, and only one aborted again. The blocking activity demonstrable in 11 of 49 women was increased after the transfusions. Subsequently five of them became pregnant, and all aborted again. We later found that these five women, who considered themselves to be in perfect health, all had serologic signs of autoimmune disease. We advise against transfusion treatment of women who habitually abort without preceding immunologic investigation, because a population of habitual aborters may contain women with yet undiagnosed autoimmune disease, who would be worse off after blood transfusion. We conclude from our results that a selected population of habitual aborters, that is, those without blocking antibody, benefits from transfusion treatment. (AM J OBSTET GYNECOL 1986;154:516-20.)

**Key words:** Habitual abortion, transfusion, autoimmune disease, mixed lymphocyte culture, blocking antibody

During the last few years, much evidence has accumulated supporting the idea that habitual abortion may be due to aberrations of the immunologic processes that are parts of normal pregnancy. Thus sharing of histocompatibility (HLA) antigens, both ABC and DR, is significantly more common among aborting couples than that expected by chance.<sup>1-3</sup>

Further, the blocking IgG antibody demonstrable in the serum of women during<sup>4</sup> and after<sup>5</sup> normal pregnancy is absent in habitual abortion.<sup>6</sup> This antibody has been shown to inhibit cell-mediated immune reactions<sup>3,6</sup> and, when bound to antigens on trophoblast or fetus, may induce fetal suppressor cells,<sup>3</sup> which is probably an important fetal defense mechanism against rejection by the mother.<sup>7</sup>

Faulk et al.<sup>8</sup> have postulated a hypothesis for the maintenance of pregnancy. They have identified two serologically defined trophoblast antigen groups, called TA<sub>1</sub> and TA<sub>2</sub>. TA<sub>1</sub>, which is present on trophoblast and

certain human cell lines, induces a cytotoxic T-cell response. TA<sub>2</sub> is demonstrable on trophoblast, lymphocytes, endothelium, and villous fibroblasts and stimulates B cells to produce blocking IgG antibody, which protects against rejection-abortion. The increased HLA compatibility between husband and wife in habitual abortion may lead to an increased HLA compatibility between mother and fetus and thereby possibly to an inadequate stimulus to production of blocking antibody. The antigen capable of initiating this antibody production is also demonstrable on leukocytes.<sup>8</sup> If this antigen is presented to the women's lymphocytes together with foreign HLA antigens, the chances of initiation of antibody production will increase. Based on this theory, transfusions<sup>9</sup> or subcutaneous injections<sup>10,11</sup> of leukocytes have been used to treat women with recurrent abortion. We have given habitually aborting women transfusions of blood cell concentrates, containing leukocytes as well as erythrocytes. Their immunologic response was determined before and after the transfusion series.

## Material and methods

**Patients.** Forty-nine women who had experienced at least three consecutive abortions were sent from all parts of Sweden for immunologic investigations. Twenty-eight were primary habitual aborters, who had had three to 11 abortions and no deliveries. The 21 women with secondary habitual abortion had been pregnant four to eight times, and three to seven of their pregnancies had resulted in abortions (see Table I). The

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cervical intraepithelial neoplasia did not reach statistical significance.

A model reconciling most seroepidemiologic and experimental findings in human cervical cancer has been outlined by zur Hausen.<sup>16</sup> Infection of normal cervical cells with specific types of papillomaviruses leads to cellular proliferation. Progression to higher degrees of cervical intraepithelial neoplasia and ultimately to invasive cancer is mediated by initiating factors such as herpes simplex virus infections and smoking. As indicated in the present study, human papillomavirus may also have a secondary promoter effect by decreasing immune surveillance through a reduction in intraepithelial Langerhans' cells. If a permissive wart virus infection persists following the development of cervical intraepithelial neoplasia, then koilocytosis is seen in association with cervical intraepithelial neoplasia and continued depletion of Langerhans' cells results in intermediate densities. If the wart virus infection becomes nonpermissive, the cytopathic effect of wart virus is lost, no koilocytosis is seen, and the presence of cervical intraepithelial neoplasia results in a marked increase in Langerhans' cell numbers.

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virus infection was compared with areas of squamous metaplasia, the decrease in Langerhans' cell numbers did not reach statistical significance.

In 12 biopsies both ectocervix and cervical intraepithelial neoplasia were included in the same biopsy. The mean Langerhans' cell density in the ectocervix was 22.6 cells per square millimeter compared with a density of 41.9 cells per square millimeter in the areas of cervical intraepithelial neoplasia. When only cervical intraepithelial neoplasia grade 3 was considered, the mean ectocervical Langerhans' cell density was 24 cells per square millimeter and the density of Langerhans' cell in the areas of cervical intraepithelial neoplasia increased to 52.2 cells per square millimeter. Although the mean Langerhans' cell density in the areas of cervical intraepithelial neoplasia was twice that in the corresponding ectocervix, the difference was not statistically significant.

### Comment

Experimental evidence suggests that Langerhans' cells may form part of an immune surveillance system preventing the development of malignancy in squamous epithelium. Agents associated with an increased incidence of skin tumors, including therapy with psoralen and long wavelength ultraviolet light,<sup>6</sup> topical administration of 9,10-dimethyl-1,2,benzanthracene,<sup>11</sup> and systemic glucocorticoids,<sup>12</sup> have been shown to result in depletion of epidermal Langerhans' cells.

The association of human papillomavirus with the development of malignancy is well documented. In patients with the genetically determined disease epidermodysplasia verruciformis, cutaneous lesions infected with human papillomavirus type 5 show a predisposition to transformation to squamous cell carcinoma.<sup>13</sup> Human papillomavirus DNA type 6 has been identified by hybridization techniques in the apparently malignant portions of two verrucous carcinomas of the anogenital region,<sup>14</sup> and there is histologic evidence that even verruca vulgaris may be associated with benign and malignant neoplasms of the skin.<sup>15</sup> Molecular hybridization has demonstrated that whereas benign condylomatous lesions of the cervix are characterized by human papillomavirus DNA types 6 and 11, neoplastic cervical lesions have been demonstrated to contain human papillomavirus DNA types 16 and 18.<sup>8</sup>

Depletion of Langerhans' cells has been demonstrated in cutaneous warts, and the present study confirms the prior observation that Langerhans' cell numbers are decreased in cervical condyloma.<sup>2</sup> When lesions of cervical condyloma were compared with normal ectocervix, the decrease in Langerhans' cell density was statistically significant. However, when compared with areas of squamous metaplasia, a more valid comparison as the majority of the lesions of cervical con-

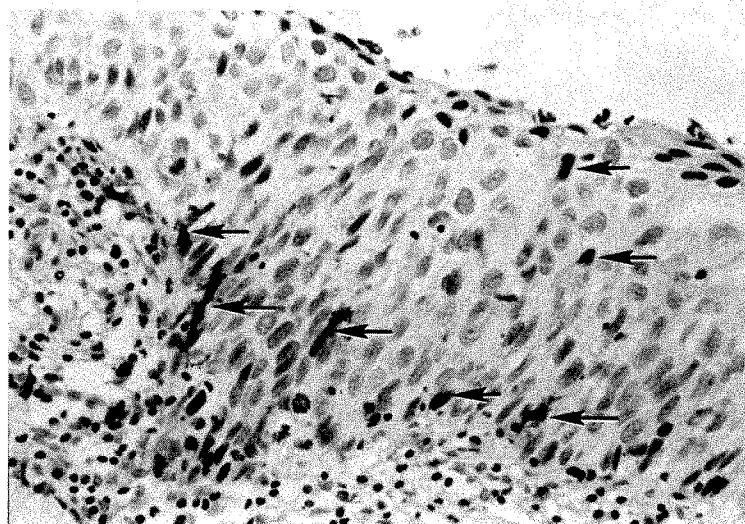
dyloma occurred in the transformation zone, Langerhans' cell density in areas of cervical condyloma was again reduced but the results did not reach statistical significance. In addition, there was a significant difference in Langerhans' cell density when areas of cervical intraepithelial neoplasia were compared with areas of cervical intraepithelial neoplasia showing histologic evidence of wart virus infection.

As proposed by Morris et al.,<sup>2</sup> such cervical Langerhans' cell depletion may be the result of a direct cytotoxic effect of wart virus on Langerhans' cells or may reflect increased transit of Langerhans' cells to regional lymphoid tissue. Although human papillomavirus DNA has been demonstrated in cervical condyloma, koilocytic dysplasia, and cervical intraepithelial neoplasia,<sup>8</sup> decreased Langerhans' cell numbers appear related to the presence of koilocytosis and viral replication rather than to viral DNA. Such depletion, through a reduction in local immune surveillance, may assist in the development of cervical cancer in lesions caused by human papillomavirus types 16 and 18.

The dramatic increase in Langerhans' cell density with cervical intraepithelial neoplasia observed by Morris et al.<sup>2</sup> and confirmed by Caorsi and Figueroa<sup>7</sup> and the present study suggests an immune response directed against neoplastic transformed cells. Langerhans' cells can handle complex antigens,<sup>5</sup> and recent work in our laboratory (unpublished) has shown that Langerhans' cells are capable of presenting cellular antigens (sheep red blood cells) to effector cells. Therefore, Langerhans' cells are potentially capable of performing a surveillance function against the emergence of neoantigens associated with malignant transformation.

The intermediate Langerhans' cell density found in koilocytic dysplasia is presumably due to the combination of wart virus-induced Langerhans' cell depletion and the increased Langerhans' cell numbers associated with cervical intraepithelial neoplasia. It is also possible, however, that the different serotypes of human papillomavirus found in cervical condyloma and koilocytic dysplasia have differing cytopathic effects on Langerhans' cell.

The increase in Langerhans' cell density in ectocervix associated with cervical intraepithelial neoplasia is as yet unexplained but is similar to our findings in normal epidermis adjacent to cutaneous in situ and invasive neoplasia (unpublished). It suggests that the factor(s) responsible for the aggregation of Langerhans' cells in areas of neoplasia is (are) also operating, to a lesser extent, in the adjacent, morphologically normal epithelium. As a result of this increase in ectocervical Langerhans' cells, when foci of cervical intraepithelial neoplasia were compared with ectocervix in the same biopsy, the increase in Langerhans' cell numbers in



**Fig. 6.** Cervical intraepithelial neoplasia grade 3. Intraepithelial Langerhans' cells are demonstrated with antibody to S100 protein (arrows). (Indirect immunoperoxidase  $\times 250$ .)

**Table I.** Langerhans' cell density in cervical lesions

	Mean Langerhans' cell density (cells/mm <sup>2</sup> )	No. in each group	<i>p</i> value* for comparison with cervical intraepithelial neoplasia	<i>p</i> value* for comparison with cervical intraepithelial neoplasia grade 3	<i>p</i> value* for comparison with cervical condyloma
Ectocervix	12.6	46	0.01	0.01	0.01
Squamous metaplasia	13.5	13	0.01	0.05	NS
Cervical condyloma	4.6	14	0.001	0.001	—
Cervical intraepithelial neoplasia	37.7	21	—	—	0.001
Cervical intraepithelial neoplasia grade 3	45	14	—	—	0.001
Koilocytic dysplasia	12.5	20	0.01	—	0.001

NS, Not significant.

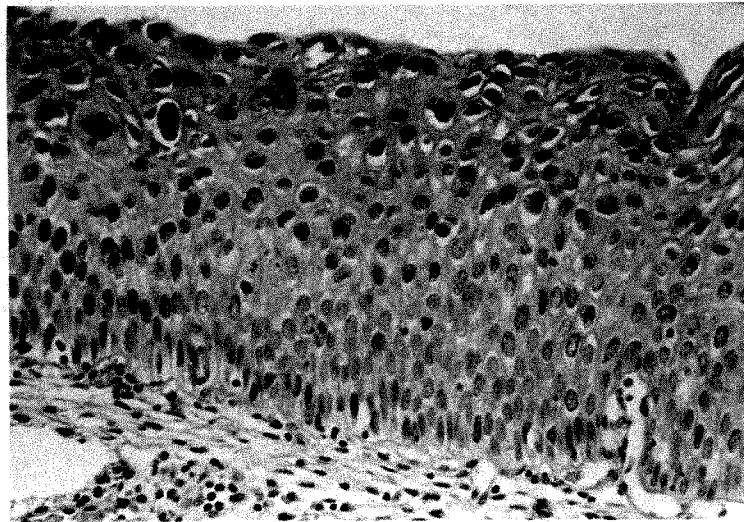
\*Significance of difference assessed by Wilcoxon rank sum test.

by diluting the primary antibody to 1:160 so as to facilitate counting of cell bodies.

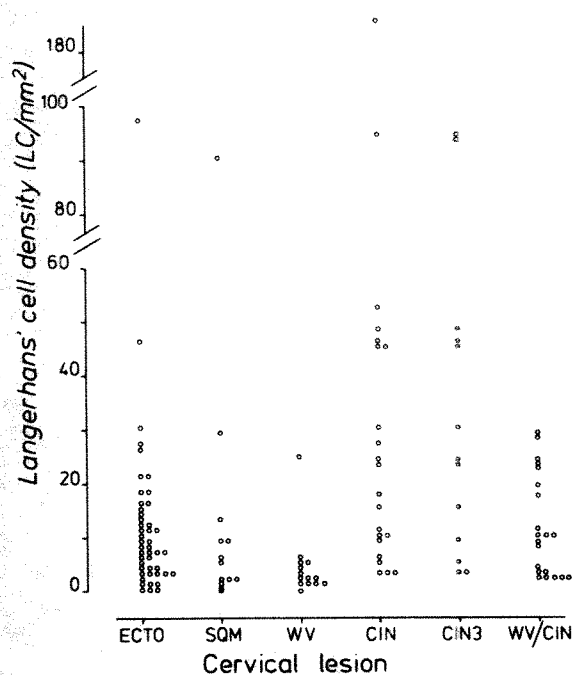
In the 79 biopsies there were 46 examples of normal ectocervix, 13 of squamous metaplasia, and 14 of cervical condyloma (uncomplicated wart virus infection). In three biopsies the wart virus infection was located in the ectocervix (Fig. 1) whereas in the remaining 11 cases wart virus infection was seen in metaplastic squamous epithelium of the transformation zone (Fig. 2). There were 21 examples of cervical intraepithelial neoplasia including 14 cases of grade 3, five of grade 2, and two of grade 1. In an additional 20 cases there was evidence of both cervical intraepithelial neoplasia and wart virus infection (Fig. 4). Wart virus was associated in four cases with cervical intraepithelial neoplasia grade 1, in 14 cases with grade 2, and in two cases with grade 3. Twenty-six biopsies included normal ectocervix alone whereas normal ectocervix was seen in the

same biopsy as cervical intraepithelial neoplasia in 12 cases, wart virus infection in three cases, squamous metaplasia in five cases, and koilocytic dysplasia in 10 cases.

The mean Langerhans' cell densities expressed as Langerhans' cells per square millimeter of epithelium and statistical evaluation of the results according to the Wilcoxon rank sum test are shown in Table I. Although there was wide variation in Langerhans' cell numbers in each lesion (Fig. 5), their numbers were reduced in cervical condyloma (Figs. 1 and 2) and increased in lesions showing cervical intraepithelial neoplasia (Fig. 6) when compared with normal ectocervix. In lesions in which cervical intraepithelial neoplasia was associated with wart virus infection, intermediate values for Langerhans' cell density were obtained. There was no difference in Langerhans' cell density between squamous metaplasia and normal ectocervix. When wart



**Fig. 4.** Koilocytic dysplasia with features of both wart virus infection and cervical intraepithelial neoplasia. No Langerhans' cells are seen in this field following staining with antibody to S-100 protein. (Indirect immunoperoxidase  $\times 250$ .)



**Fig. 5.** Langerhans' cell density in normal ectocervix (ECTO), metaplastic squamous epithelium of the transformation zone (SQM), cervical condyloma (WV), all grades of cervical intraepithelial neoplasia (CIN), cervical intraepithelial neoplasia grade 3 (CIN 3), and in koilocytic dysplasia (WV/CIN).

0.05% 3,3'-diaminobenzidine in 40 ml of phosphate-buffered saline solution to which 2 drops of hydrogen peroxide were added. The preparations were then washed for 5 minutes in running tap water, counterstained with Mayers' hematoxylin, dehydrated, cleared in xylene, and mounted in Eukitt.

**Reagents.** Normal swine serum and peroxidase-con-

jugated swine antirabbit immunoglobulins were obtained from Dakopatts A/S, Glostrup, Denmark. All sera, except the normal swine serum, were diluted with a 1% solution of ovalbumin grade 3 (Sigma Chemical) in phosphate-buffered saline solution.

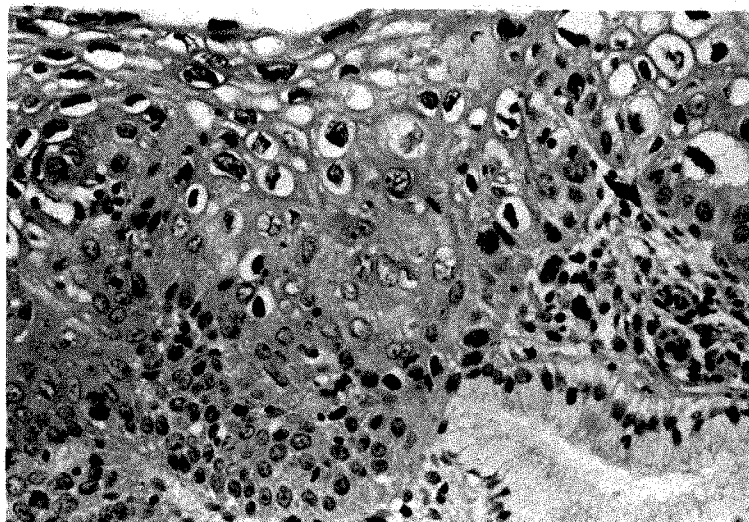
For optimal staining on test sections the anti-S100 antibody was diluted to 1:160. This resulted in good staining of Langerhans' cell nuclei and cell bodies with decreased staining of dendritic processes and a clean background. Negative controls used in the procedure included normal rabbit serum and omission of the primary antibody. Normal skin was used as a positive control.

**Assessment of Langerhans' cell density.** Langerhans' cells were counted in vertical cervical sections stained with anti-S100 antibody and visualized by light microscopy. Epithelial areas contiguous with the histopathologic areas yielding the diagnosis and Langerhans' cell counts were measured from projected tracings with use of a Bioquant II basic measuring program volume 185 (R and M Biometrics, Nashville, Tennessee) for an Apple IIe computer, taking into account magnification factors. Langerhans' cell densities in the lesions studied were compared according to the Wilcoxon rank sum test.<sup>10</sup> A p value of  $<0.05$  was regarded as statistically significant.

## Results

Indirect immunoperoxidase staining of the paraffin sections with antibody to S100 protein revealed the characteristic dendritic morphologic form of intraepithelial Langerhans' cells (Fig. 3). Both nuclear and cytoplasmic staining were present. Intensity of staining of the dendritic processes was deliberately decreased



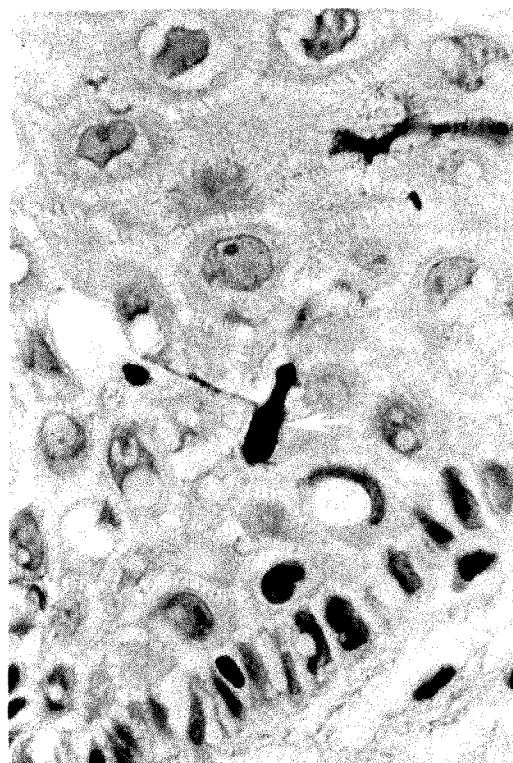


**Fig. 2.** Cervical wart virus infection in metaplastic squamous epithelium. An endocervical gland is present beneath the surface epithelium. No Langerhans' cells are seen following staining with antibody to S100 protein. (Indirect immunoperoxidase  $\times 250$ .)

intraepithelial neoplasia associated with histologic evidence of cervical condyloma. Histologic features indicative of cervical condyloma include koilocytosis, condensation of peripheral cytoplasm, nuclear irregularity, and binucleation (Fig. 1). No cases of exophytic condylomatous cervical condyloma were included. Squamous metaplasia was diagnosed when squamous epithelium was present overlying endocervical glands (Fig. 2) or when there was an irregular epithelial-stromal interface, suggesting that metaplastic squamous epithelium had replaced endocervical glands. Criteria for diagnosis of cervical intraepithelial neoplasia and koilocytic dysplasia were those outlined by Fletcher.<sup>9</sup>

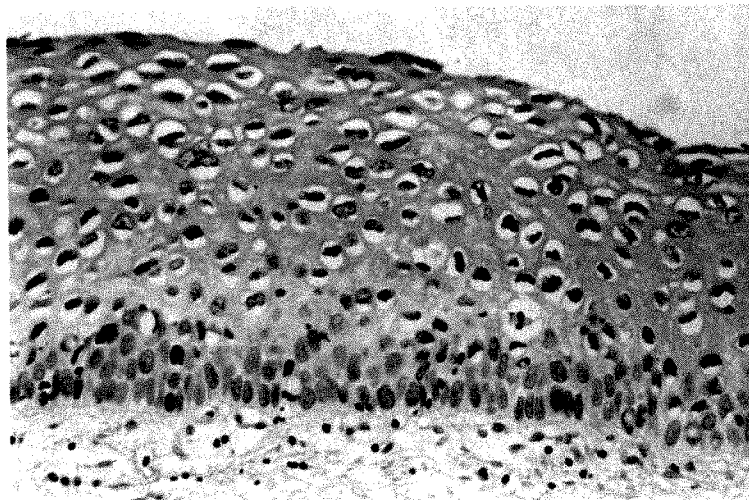
**Indirect immunoperoxidase method.** Tissue sections, 3 to 5  $\mu\text{m}$  thick, were floated on a gelatin and dichromate water bath at approximately  $45^{\circ}\text{C}$ , mounted in a 1% solution of Aquadhere on cleaned glass slides, and left uncovered at room temperature overnight. Sections were then incubated at  $37^{\circ}\text{C}$  with 0.05% protease type VII (Sigma Chemical Co., St. Louis, Missouri) in phosphate-buffered saline solution, pH 7.4, for 5 minutes.

Enzyme digestion was terminated with cold running tap water for 5 minutes and the slides were washed with phosphate-buffered saline solution for 3 minutes. Sections were then exposed to normal swine serum, diluted in a 1:5 ratio in phosphate-buffered saline solution for 5 minutes, and drained without washing. A 30-minute incubation with rabbit anti-S100 antibody (provided by Dr. G. Rowden, Dalhousie University) followed, and the sections were then washed with two changes of phosphate-buffered saline solution for 10 minutes. Blocking of endogenous peroxidase activity with hydrogen peroxide and methanol was found to



**Fig. 3.** Ectocervical intraepithelial Langerhans' cell stained with antibody to S100 protein (arrow). (Indirect immunoperoxidase  $\times 1000$ .)

be unnecessary. Sections were next incubated with a 1:50 dilution of peroxidase-conjugated swine anti-rabbit serum for 30 minutes and washed in two changes of phosphate-buffered saline solution for 10 minutes. The antigen-antibody reaction was revealed by incubating sections for 5 minutes in a fresh solution of



**Fig. 1.** Ectocervical squamous epithelium with histologic features indicative of wart virus infection including cytoplasmic vacuolation, peripheral condensation of cytoplasm, nuclear irregularity, and binucleation. No Langerhans' cells are seen following staining with antibody to S100 protein. (Indirect immunoperoxidase  $\times 250$ .)

(Ia in mouse, HLA-DR in human) on their cell membranes. Additional markers for Langerhans' cells in man include antibody to the nervous system protein S100 and the monoclonal antibody OKT6.<sup>3</sup>

Recent studies have shown that Langerhans' cells play a crucial role in local cutaneous defense as antigen-presenting cells and are responsible for the induction of contact sensitivity reactions.<sup>4</sup> In addition to being more effective than monocytes at presenting small, simple antigens to T cells, Langerhans' cells have a similar capacity to present complex antigens such as herpes simplex virus.<sup>5</sup> Friedmann<sup>6</sup> has demonstrated that in psoriasis patients treated with 8-methoxypsoralen and long wavelength ultraviolet irradiation, there is a marked reduction in the number of epidermal Langerhans' cells. Thus it has been postulated that prolonged exposure of the skin to ultraviolet light inactivates the Langerhans' cells and thereby reduces immune surveillance in the skin.<sup>4</sup> The impaired recognition of foreign antigens which then follows may allow the outgrowth of either virally transformed or spontaneously transformed malignant cells to occur more easily.

In a recent qualitative study involving a small number of cases it was shown that cervical wart virus infection resulted in depletion of cervical epithelial Langerhans' cells, and the possibility was raised that this phenomenon may indirectly contribute toward the risk of malignant transformation.<sup>2</sup> In the same study it was found that Langerhans' cell numbers were increased in areas of intraepithelial neoplasia, suggesting a specific immune response directed against neoplastically transformed cells. With use of a zinc-iodide-osmium technique Caorsi and Figueroa<sup>7</sup> have also demonstrated a

3.5-fold increase in Langerhans' cell density in three cases of cervical intraepithelial neoplasia.

In the present study 79 cervical punch biopsies were examined with antibody to S100 protein to determine the quantitative distribution of Langerhans' cells in cervical wart virus infection (cervical condyloma), in cervical intraepithelial neoplasia, and in cervical intraepithelial neoplasia with koilocytotic features (koilocytic dysplasia). Cervical condyloma is characterized by nuclear polyploidy, absence of abnormal mitoses, and human papillomavirus DNA types 6 and 11. These lesions tend to regress spontaneously or to not recur following treatment. The available evidence suggests that koilocytic dysplasia, on the other hand, tends to persist and has a greater risk of progression to high grades of cervical intraepithelial neoplasia. It is associated with nuclear aneuploidy, contains abnormal mitotic figures, and has been demonstrated by molecular hybridization to be associated with human papillomavirus DNA types 16 and 18.<sup>8</sup>

#### Material and methods

Routine blocks of 79 cervical punch biopsies were obtained from the files of the Royal Hobart Hospital Pathology Department. All had been fixed in 10% buffered formalin for 8 to 24 hours and, after routine processing, embedded in paraffin wax. The blocks had been stored for periods up to 6 years. Sections were stained with hematoxylin and eosin and with antibody to S100 protein with use of an indirect immunoperoxidase technique.

**Diagnosis.** The cervical lesions studied included squamous metaplasia, varying grades of cervical intraepithelial neoplasia, cervical condyloma, and cervical

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## Quantitative assessment of Langerhans' cells in human cervical intraepithelial neoplasia and wart virus infection

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Langerhans' cell density was assessed quantitatively in cervical wart virus infection (cervical condyloma), cervical intraepithelial neoplasia, and koilocytic dysplasia with use of an antibody to S100 protein and an indirect immunoperoxidase technique. When compared with normal ectocervix, Langerhans' cell density was significantly decreased in cervical wart virus infection and significantly increased in cervical intraepithelial neoplasia. In koilocytic dysplasia, intermediate Langerhans' cell densities were obtained. In addition to being increased within the lesions of cervical intraepithelial neoplasia, Langerhans' cell density was increased in the adjacent normal ectocervix. Human papillomavirus, by reducing intraepithelial Langerhans' cell density, may decrease local immune surveillance and thus have a promoter effect in the development of cervical cancer. Following the development of cervical intraepithelial neoplasia the increase in intraepithelial Langerhans' cell density suggests a specific immune response directed against neoantigens associated with malignant transformation. If a permissive wart virus infection persists after transformation to cervical intraepithelial neoplasia (koilocytic dysplasia), continued depletion of Langerhans' cells results in intermediate densities. (*AM J OBSTET GYNECOL* 1986;154:509-15.)

**Key words:** Immunoperoxidase, S100 protein, cervical intraepithelial neoplasia, wart virus infection, Langerhans' cells

Langerhans' cells were initially described in the human epidermis by Paul Langerhans in 1868. These cells constitute a small subpopulation (3% to 8%) of epidermal cells and are characterized ultrastructurally by dendritic processes and a unique organelle, the Birbeck

granule.<sup>1</sup> Subsequently, Langerhans' cells have been described in lymph nodes, thymus, dermis, and cervix.<sup>1,2</sup> Histochemical methods used to identify Langerhans' cells include the demonstration of membrane-associated adenosine triphosphatase.<sup>1</sup>

Langerhans' cells are now considered a population of bone marrow-derived dendritic cells that migrate to the dermis and epidermis. The surface marker characteristics of Langerhans' cells are similar to those of the monocyte-macrophage series. They have receptors for the Fc portion of IgG and the complement component C3b, and they express class II MHC antigens

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found similar relationships between overweight and low weight gain but are relatively certain that low gains were not due to prescribed dieting. Almost all cases of low weight gain or loss were assessed by a nutritionist and these women were counseled to attempt to increase their weight gains. A higher percentage of the very overweight women gained >20 kg even after cases of pregnancy-induced hypertension, a disorder associated with excessive weight gain, were excluded.

Because our results indicate that pregnancy weight gain for overweight women does not have an impact on infant birth weight, it is tempting to consider use of a weight reduction diet for these women during pregnancy—a popular approach before 1970. Since our study did not include any measures of diet or nutritional status, our data cannot be used to address this issue. Also, we were unable to evaluate the incidence or effect of acetonuria, since these data were not available.

Several reports of dieting obese pregnant women have been published with differing conclusions. Broberg et al.<sup>19</sup> used a 1800 to 2000 kcal diet and reported that dieted women gained only 6 kg in the last 24 weeks of pregnancy as compared to undieted women who gained 14 kg. They claimed there were no ill effects of the dietary restriction for the mothers or the neonates. Another study placed 90 overweight pregnant women on a 1500 kcal diet for the last 10 weeks of pregnancy; although weight gain was less in the dieting than in the nondieting group of 90, the authors indicated that the lack of gain was due to decreased water retention (detected by a decreased plasma volume) in the dieting group compared to the nondieting group and not due to decreased fat.<sup>20</sup> This is not surprising, since it has been hypothesized that most of the maternal fat storage during pregnancy occurs during the first and second trimesters,<sup>21</sup> but it suggests that whatever maternal fat was stored was not mobilized to meet the energy needs for fetal growth. This study did find that 25% of the neonates in the dieting obese group weighed less than the twenty-fifth percentile compared to 17% of the neonates in the control obese group. The authors considered this to be an adverse effect of the diet but did not test for statistical significance. Jacobson et al.<sup>22</sup> also dieted overweight pregnant women with use of a 1500 calorie diet. The control group was made up of overweight women who were unable or unwilling to comply with the dietary restriction. The dieting group experienced a mean net loss of 5 kg as compared with the 4.3 kg increase for the control group; birth weights for both groups were adequate although the infants of the control group were bigger (3634 compared to 3407 gm). Follow-up 6 months after delivery indicated that most of the women continued to be overweight and that the women in the dieting group, by and large, had

regained any weight they lost as a result of dieting during pregnancy. These limited results with differing, nonrandom study designs suggest that we need to learn more about the potential benefits and risks for mother and neonate before seriously considering a standard diet for all pregnant overweight women.

This study confirms the association between maternal weight gain and birth weight for women with low, average, or moderately elevated prepregnancy weights and supports published recommendations<sup>7-9</sup> for total weight gain during pregnancy. It provides additional evidence for the recently published concept by Rosso<sup>23</sup> that weight gain recommendations be based on prepregnancy weight for height.

In this sample, maternal weight gain was not associated with infant birth weight for very overweight women. These findings suggest that a standard recommendation for a minimum amount of maternal weight gain for very overweight pregnant women is unnecessary and inappropriate. At the same time the weight gains of these obese women who experienced good pregnancy outcomes were extremely variable, ranging from none to >40 kg, and all levels of weight gain were associated with infants of adequate size. We suggest that for obese pregnant women the focus of attention should be on individualized assessment of fetal growth, dietary quality, and exercise patterns to assure nutritional adequacy rather than on weight gain alone. This approach holds the potential for ensuring a healthy pregnancy outcome without increasing maternal obesity.

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**Table IV.** Multiple regression analysis of effect of weight gain on birth weight (live births in term pregnancies)

	Weight gain regression coefficient	SE	Total R <sup>2</sup>	Change in R <sup>2</sup>	
				Covariables*	Weight gain
All weights	15.9	1.6	0.24	0.19	0.05†
Underweight	25.9	6.4	0.18	0.14	0.05‡
Ideal weight	28.3	2.3	0.27	0.19	0.08†
Moderately overweight	17.8	2.7	0.26	0.21	0.04§
Very overweight	2.2	4.4	0.14	0.14	0.001

\*Maternal age, race, socioeconomic status, parity, cigarettes per day, pregravid body mass, gestational age.

†p &lt; 0.00001.

‡p &lt; 0.0001.

§p &lt; 0.0002.

**Table V.** Multiple regression analysis of weight gain on birthweight (live births in uncomplicated term pregnancies)

	Weight gain regression coefficient	SE	Total R <sup>2</sup>	Change in R <sup>2</sup>	
				Covariables*	Weight gain
All weights	22.1	1.6	0.25	0.20	0.05†
Underweight	30.7	4.2	0.25	0.17	0.08†
Ideal weight	28.9	2.3	0.27	0.19	0.08†
Moderately overweight	19.0	2.8	0.27	0.23	0.04†
Very overweight	4.3	4.7	0.17	0.17	0.001

\*Pregnancies uncomplicated by diabetes or hypertensive disorders. Adjustable for maternal age, race, socioeconomic status, parity, cigarettes per day, pregravid body mass, gestational age.

†p &lt; 0.00001.

these pregnancies than on others by increasing birth weight and bringing it into a normal range.

It is interesting to note that the mean weight gain during pregnancy in this recent sample was 15.3 kg, which is higher than the usually reported 10 to 12 kg. We feel this reflects the more recent recommendations, which encourage weight gain during pregnancy. The women in this sample were also older (mean age, 27.3 years) than average, which reflects the recent trend in delayed childbearing. Although we controlled for race in the analysis, the racial heterogeneity of this sample is important to consider, since our findings may be relevant only to a population with the same racial characteristics as the one we have described here. However, despite the differences in weight gain, maternal age, and racial parameters in this sample, the finding that there is a significant linear relationship between maternal weight gain and birth weight for women who are not excessively overweight confirms earlier studies and supports current recommendations. This was also true when the data were analyzed after excluding women with diabetes and hypertensive disorders.

Weight gain during pregnancy had no significant effect on birth weight for the very overweight women—those who weighed more than 135% of the standard

before pregnancy. This finding contradicts other reports that maternal weight gain produced a measurable increase in birth weight for infants born to overweight women. Luke et al.<sup>13</sup> reported that weight gain increased birth weight in low-income overweight black pregnant women, and Gormican et al.<sup>12</sup> found a statistically significant linear relationship between weight gain and birth weight in middle-class overweight white women. Each of these studies used 120% of standard weight for height as the definition of overweight; we further stratified our sample and examined women of >135%. It was in this extreme group that weight gain and birth weight were poorly correlated. Several other studies have also reported that while weight gain and birth weight were associated for women whose pregravid body weights were close to average, heavier women (usually greater than 160 to 180 pounds at conception) delivered average to large size infants even with low gains or weight loss.<sup>4-6, 15</sup>

Overweight women were heterogeneous with regard to the amount of weight they gained during pregnancy, with a higher percentage gaining both more and less than average. In earlier studies, increased rates of low weight gain in the overweight women were believed to be due to dietary restriction by physicians.<sup>4-6</sup> We

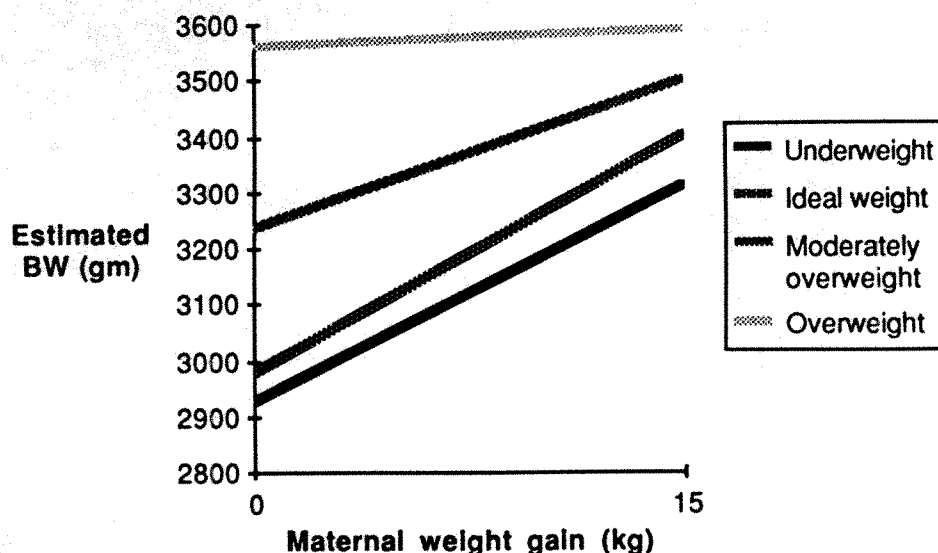


Fig. 1. Birth weight of live-born infants at term by prepregnancy body mass and weight gain, adjusted for maternal age, race, parity, socioeconomic status, cigarette consumption, and gestational age ( $n = 2964$ ).

regression analysis on the same sample examined the relationship between maternal weight gain and birth weight: there is a highly significant and linear ( $Y = (\text{covariates}) + 20.1x - 1517$ ,  $F = 177.1$ ,  $p < 0.00001$ ) 20.1 gm increase in birth weight for a 1 kg increase in maternal weight gain after removing the effects of maternal age, race, parity, socioeconomic status, cigarette consumption, gestational age, and prepregnancy body mass. The  $R^2$  for the entire model was 0.24, indicating that all the variables studied explained 24% of the variation in birth weight. Prepregnancy body mass explained 1.5% of this variation and maternal weight gain explained 4.6%—more than 20% of the variance we were able to explain.

To determine whether maternal weight gain affected birth weight differently at different levels of prepregnancy body mass, a multiple regression analysis was carried out separately for each weight group. Results are illustrated in Fig. 1 and summarized in Table IV.

The effect of maternal weight gain on birth weight was statistically significant for the underweight, ideal weight, and moderately overweight women; each kilogram of maternal weight gain increased infant birth weight by 25.9, 28.3, and 17.8 gm, respectively. In the very overweight group the slope of the line representing the effect of maternal weight gain on infant birth weight was not different from 0 and therefore not statistically significant.

Table IV presents the proportion of variation in birth weight explained by the total model (pregnancy body mass, maternal age, race, parity, socioeconomic status, cigarette consumption, gestational age, and weight gain) and by weight gain alone after removing effects

of the other variables. The total model explained a higher proportion of the variation in birth weight for the ideal weight and moderately overweight groups than for the underweight and very overweight groups. The amount of variation attributable to weight gain alone ranged from 8.0% for the ideal weight group to 0.1% for the overweight group.

The results of identical multiple regression analyses on the "low-risk" sample are found in Table V. There is no difference in the relationships between prepregnancy body mass, weight gain, and birth weight in the sample with all the cases of diabetes and hypertensive disorders removed, as compared with the total sample. Weight gain has a significant influence on birth weight for all weight groups except for the overweight group.

#### Comment

Results from this study confirm earlier reports that birth weight increases as pregravid body weight increases. Mean birth weight differed by more than 300 gm between the underweight and very overweight groups.

In this population, maternal weight gain significantly increased birth weight for women with body mass indices of  $\leq 135\%$  of the standard before pregnancy, with an equal effect on underweight and ideal weight women and slightly less for moderately overweight women. It had been previously reported that pregnancy weight gain had a more important impact on birth weight for underweight women than for women of average weight.<sup>18</sup> We also found that since underweight women had babies with the lowest mean birth weights, maternal weight gain had a greater impact on

**Table III.** Characteristics of the weight groups with deliveries after 36 weeks and live births

	<i>Underweight</i> ( <i>n</i> = 268)		<i>Ideal weight</i> ( <i>n</i> = 1535)		<i>Moderately overweight</i> ( <i>n</i> = 901)		<i>Very overweight</i> ( <i>n</i> = 224)	
	<i>Mean</i>	<i>SD</i>	<i>Mean</i>	<i>SD</i>	<i>Mean</i>	<i>SD</i>	<i>Mean</i>	<i>SD</i>
Pregravid weight (kg)	46.1	5.5	53.3	5.4	62.8	7.1	86.8	17.1
Height (cm)	161.7	8.3	160.6	6.7	159.6	7.2	160.2	7.3
Body mass index	17.4	0.92	20.6	1.1	24.6	1.6	33.6	5.5
Weight gain (kg)	14.3	4.4	15.2	4.7	15.2	5.3	14.1	7.8
Birth weight (gm)	3290	497	3414	477	3521	504	3593	514
Maternal age (yrs)	27.4	5.5	27.0	5.3	27.7	5.7	27.8	5.4
Primigravida (%)	58.0		59.5		44.1		36.2	
Pregravid weight relative to ideal (%)	<90		90-119		120-135		>135	
Percent of sample	10		52		31		7	

based on the values proposed by Garrow<sup>17</sup> to reflect the 1959 Metropolitan Insurance Standards of ideal weight for a woman of medium frame and are presented in Table I.

Low maternal weight gain was defined as total pregnancy gain of <7 kg; excessive gain was defined as a gain at term of >20 kg.

**Statistical analysis.** Multiple regression analysis was used to assess the effect of prepregnancy body mass and weight gain on birth weight. Other variables that were significantly associated with birth weight and either prepregnancy body mass or weight gain in univariate analysis were also included as independent variables in the multiple regression model. After carrying out analysis on the entire study group, the sample was stratified by prepregnancy weight and a separate multiple regression analysis was run on the underweight, average weight, moderately overweight, and very overweight groups.

A second set of multiple regression analyses was then carried out on a "low-risk" sample, which was created by excluding all cases of maternal diabetes (gestational and pregestational), hypertension, and pregnancy-induced hypertension. After exclusion of these conditions the low risk sample provided 2767 cases for analysis.

Results were considered significant if the two-tailed *p* value was less than 0.05.

## Results

Table II provides descriptive detail on the entire sample, and Table III reports selected characteristics for each weight group. Mean maternal age for the sample was 27.3 with a range of 14 to 46 years. Maternal weight gain during pregnancy ranged from a loss of 2 kg to a gain of 44 kg with a mean of 15.2 kg. Only eight women lost or gained no weight during pregnancy; two were in the underweight group, one was in the moderately overweight group, and five were in the very

overweight group. The rate of low gain was 4% for the entire sample; the rate for very overweight women was 13.6%. Thirteen percent of the entire sample gained >20 kg; there was an increase in the rate of high weight gain as pregravid body mass increased, with 18% of the overweight women falling into the high weight gain category.

Multiple regression analysis allows the estimation of the association between an outcome (dependent variable) and various predictor (independent) variables. This test describes relationships in the data in several ways. The regression coefficient describes the amount of change (in grams) in birth weight associated with a one-unit change in each independent variable after removing the effect of the other independent variables. The *F* ratio and its *p* value tests whether there is a significant association between the independent variable and birth weight. The *R*<sup>2</sup> is the proportion of the variance in birth weight explained by all the independent variables entered into the model. The *R*<sup>2</sup> change is the proportion of variance in birth weight specifically related to a particular variable after it is entered into the model. We used multiple regression analysis to evaluate the effect of prepregnancy body mass and weight gain on birth weight, while removing the effects of maternal age, race, socioeconomic status, cigarette smoking, parity, and gestational age.

The first multiple regression analysis was performed on the entire sample of 2946 births. After adjusting for maternal age, race, parity, weight gain, socioeconomic status, cigarette consumption, and gestational age, there is a statistically significant linear relationship between prepregnancy body mass and birth weight ( $Y = (\text{covariates}) + 15.9x - 1517$ ,  $F = 68.1$ ,  $p < 0.00001$ ). The regression coefficient is 15.9, indicating that a one-unit increase of prepregnancy body mass is associated with a statistically significant 15.9 gm increase in birth weight after removing the effects of the covariables and maternal weight gain. The second

**Table I.** Definition of pregravid body mass

	% of standard weight	Quetelet index
Underweight	<90	<18
Ideal weight	90-120	19-22
Moderately overweight	121-135	23-28
Very overweight	>135	>28

Concern about the relatively high rates of low birth weight deliveries in the United States led to studies in the 1960s and 1970s that reported a linear relationship between maternal weight gain and birth weight<sup>4-6</sup>; since the 1970 publication of *Maternal Nutrition and the Course of Pregnancy* by the National Academy of Sciences,<sup>7</sup> women have been encouraged to "eat to appetite" and to gain a minimum of 24 pounds (11 kg) during pregnancy.<sup>8,9</sup> Birth weight has also been correlated with prepregnancy weight and height. Underweight women tend to deliver infants with lower mean birth weights, and heavier women tend to deliver infants with higher mean birth weights.<sup>4-6,10</sup> Several studies have found a linear relationship between birth weight and maternal weight gain at all levels of prepregnancy weights,<sup>11-13</sup> while others report that as prepregnancy weight increases, the importance of maternal weight gain diminishes.<sup>4-5,14</sup> Thus, although it is clear that prepregnancy weight and maternal weight gain exert some influence on birth weight, a question remains regarding the influence of maternal weight gain, given differing prepregnancy weights. Specifically, does a lower weight gain in an overweight woman create the same result in birth weight as it does in an underweight woman? This question is relevant since "inadequate weight gain" has been reported to be a complication of pregnancies in overweight women.<sup>15</sup>

Since most of the data now published were collected before the National Academy of Sciences' report (under the prevailing philosophy that restricted maternal nutritional intake and weight gain), we decided that an analysis of more recent data would provide insight into weight relationships given unrestricted diet and weight gains. Since few earlier studies simultaneously controlled for socioeconomic status, maternal age, maternal race, parity, cigarette consumption, and gestational age (all known to be associated with birth weight), we included these variables in our analysis to remove their influence and allow exploration of the relationship between pregravid body weight, pregnancy weight gain, and birth weight.

#### Material and methods

The Department of Obstetrics, Gynecology and Reproductive Sciences' Perinatal Database prospectively records data on pregnancy course and outcome for all

**Table II.** Characteristics of the study population (N = 2948)

	Mean	Standard deviation
Maternal age (yr)	27.3	5.5
Pregravid weight (kg)	58.1	12.3
Height (cm)	160.3	7.1
Body mass	22.5	4.3
Weight gain (kg)	15.2	5.3
Birth weight (gm)	3448.4	496.7
Primiparous (%)	53.0	
Low socioeconomic status (%)	29.0	
Race (%)		
Hispanic	11.3	
White	50.3	
Black	10.4	
Asian	17.3	
Other	10.7	
Cigarette smokers (%)	16.0	
> 1 pack per day	3.0	
Underweight (%)	9.7	
Ideal weight (%)	52.0	
Moderately overweight (%)	30.7	
Very overweight (%)	7.6	

deliveries occurring at Moffitt Hospital of the University of California, San Francisco. Routine prenatal care includes the provision of nutrition education and counseling with an emphasis on adequate, unrestricted diets and total weight gain of at least 24 pounds (11 kg). Data on each patient are recorded at the first antenatal visit, at delivery, at maternal discharge, and at infant discharge. After verification for accuracy and internal consistency, the more than 300 variables are computerized.

The sample consisted of all women with singleton pregnancies of  $\geq 37$  weeks that produced a live infant in which deliveries were at Moffitt Hospital between September, 1980, and December, 1983. When the same woman experienced more than one pregnancy during the study period, only the first pregnancy was included in the analysis. To achieve a sample that is typical of an average group of women seeking prenatal care, certain cases were excluded: maternal transfers, transports, intrauterine transfusions, and fetal surgeries were eliminated to reduce bias caused by the inherent high-risk nature of these pregnancies. According to these criteria, 2946 women were available for study. Data on race, parity, maternal age, number of cigarettes smoked per day, prepregnancy weight, and height were obtained by interview at the first antenatal visit. Socioeconomic status was based on maternal education and family income.<sup>16</sup> Pregnancy weight gain was calculated by subtracting the stated prepregnancy weight from the measured weight at the last prenatal visit, which was almost always within 1 week of delivery. Birth weight was measured immediately after delivery.

Prepregnancy body mass was computed with use of the Quetelet Index (weight in kilograms divided by height in cm<sup>2</sup>). The weight classifications used were



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## Prepregnancy weight, weight gain, and birth weight

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The effect of maternal weight gain on birth weight in 2946 live births with delivery after 37 weeks' gestation was studied at Moffitt Hospital, University of California (San Francisco), between September, 1980, and December, 1983. The sample was stratified into four categories according to prepregnancy weight for height with use of a body mass index. To study the effect of maternal weight gain on infant birth weight, multiple regression analysis, controlled for selected covariables, was carried out on the entire sample and on each prepregnancy weight group. For the entire sample, both pregravid body mass and weight gain significantly influenced birth weight. For the underweight, ideal weight, and moderately overweight women, each kilogram of maternal weight gain significantly increased birth weight. This study supports recent evidence for the association between maternal weight gain and birth weight, but only for women whose prepregnancy weights are 135% of ideal or less. These results suggest that recommendations for a minimum weight gain for obese women are unnecessary. (*AM J OBSTET GYNECOL* 1986;154:503-9.)

**Key words:** Obesity, pregnancy, birth weight, weight, body weight

Over the last 60 years there have been significant changes in the recommendations made to women about weight gain during pregnancy. In the 1920s, efforts were made to limit maternal gains to not more than 15 pounds (6.8 kg) in an attempt to promote easier labors

and preserve the woman's figure after birth.<sup>1</sup> During the 1940s the association between excessive weight gain and preeclampsia was believed to be causative,<sup>2</sup> and weight restriction was a common goal. This approach was still in vogue 20 years later, as this section from the 1966 edition of *Williams' Obstetrics*<sup>3</sup> illustrates:

Excessive weight gain in pregnancy is highly undesirable for several reasons; it is essential to curtail the increment in gain to 25 pounds at most or preferably 15 pounds. The experienced obstetrician is convinced of the complications, both major and minor, caused by excessive weight gain in pregnancy. Although restriction of the gain in weight to 20 pounds may be difficult in many cases, requiring careful dietary control and discipline, it is a highly desirable objective.

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intrauterine device users unless there was more than one sexual partner. Our data show that the prevalence of other types of sexually transmitted disease was significantly increased in users versus nonusers ( $p = 0.01$ ) paralleling the increase in residues and positive titers. If the intrauterine device is responsible for increasing the prevalence of upper tract disease after lower tract exposure, it must also be responsible for increasing the attack rate of human papilloma virus infections, genital herpes, mycoplasma-ureaplasma infections, and the sexually transmitted vaginitides, since all are seen with increased frequency in intrauterine device users. The possibility of both increased exposure and increased attack rates cannot be excluded. Further studies will be necessary to clarify this point.

In our study the incidence of residues increased as the chlamydial antibody titer level increased. At titers of  $\geq 512$ , residues were seen in nearly all patients. Similar results were found by Punnonen et al.<sup>15</sup> and Gump et al.<sup>16</sup>

Prior studies have shown a greater risk of tubal infertility in Dalkon Shield users when compared to those patients using other types of intrauterine devices.<sup>4,5</sup> We noted no difference in residue frequency with any specific intrauterine device if chlamydial antibody titer was used for comparative grouping; however, the number in each group was too small for statistical significance.

The prevalence of ectopic pregnancy was increased in patients with residues. If the antibody titer was  $< 512$ , there was no difference in users compared to nonusers. However, in intrauterine device users with a titer of  $\geq 512$ , there was a 2½-fold increase in ectopic pregnancy frequency when compared to the nonuser group.

Gump et al.<sup>16</sup> found positive cervical chlamydial cultures infrequently in their patients even if the chlamydial antibody titer were positive. Our data confirm this finding. Overall, only 4% to 7% of cultures and smears were positive for *Chlamydia trachomatis*. Positive tests were found more frequently in those patients with positive antibody titers than in those with negative titers. It would appear that chlamydial cervical cultures and smears are of little benefit to the clinician in the diagnosis of residues of chlamydial upper tract disease as well as chronic upper tract chlamydial infection.

Particularly disturbing to us was the fact that patients with inflammatory residues in both groups II and III (positive titer groups) frequently had no history of an illness or procedure which could explain the presence of postinflammatory tubal damage. Approximately two thirds of patients in group II and three fourths of patients in group III, both users and nonusers, had a negative history for explainable causes and could be presumed to have had a silent infection. The occurrence of a sexually transmitted acute inflammatory process that is asymptomatic with such frequency has grave

prognostic implications for successful epidemiologic control and eventual eradication of that disease.

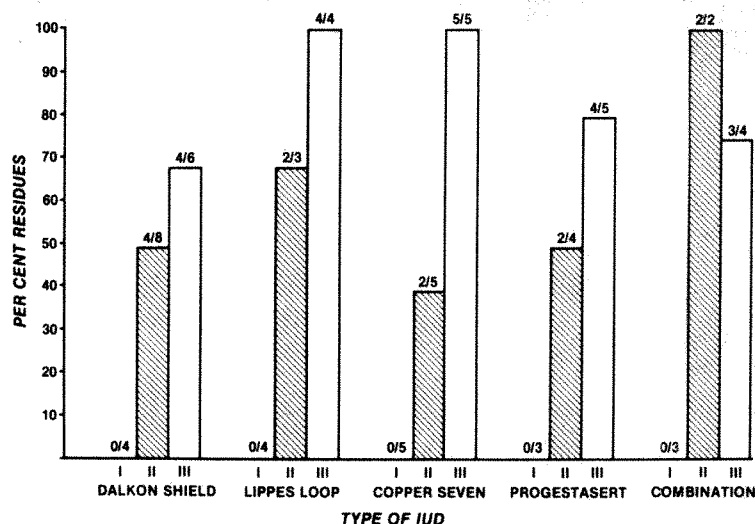
Chlamydial tubal infection, in contrast to gonococcal salpingitis, may be a true chronic active infection. It may be accompanied by negative endocervical cultures and absence of clinical symptoms.<sup>13,14</sup> Intracellular chlamydial organisms may persist in the pelvis for extremely long periods of time and continue to produce silent progressive tubal damage unless treatment with appropriate antibiotics is instituted.<sup>13</sup> We are continuing our study of *Chlamydia trachomatis* in relation to tubal infertility. Preliminary results would indicate that preoperative treatment with tetracycline may significantly reduce the frequency of ectopic pregnancy following tubal reconstructive microsurgery for chlamydial residues. This will be the subject of a future report.

In summary, intrauterine device users have adnexal inflammatory residues and tubal infertility more frequently than nonusers. Users also have positive chlamydial antibody titers more frequently than nonusers, and they have other types of sexually transmitted diseases more frequently than nonusers. However, users and nonusers have the same prevalence of residues at the same level of chlamydial antibody titer. Acute chlamydial salpingitis may be subclinical or silent in approximately two thirds to three fourths of cases. Chlamydial infection, after an initial bout of acute salpingitis, may become a chronic intrapelvic infection that can persist indefinitely until appropriate antibiotic therapy is administered. *Chlamydia trachomatis* is most probably the hidden variable in the intrauterine device/pelvic infection/residue puzzle. For this reason future studies dealing with intrauterine device-related acute salpingitis, ectopic pregnancy, tubal infertility and postinflammatory residuals should address evidence for or against current or prior chlamydial infection.

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**Fig. 3.** Graph illustrating the prevalence of residues of pelvic inflammatory disease in intrauterine device users by titer groups as related to the type of intrauterine device used. The number of patients in each group is too small to achieve statistical significance.

**Table V.** Prevalence of unexplained (silent) residues in intrauterine device users and nonusers by titer group

	Titer group					
	I		II		III	
	n	%	n	%	n	%
Users	0/5	0	11/19	57.9	19/25	76
Nonusers	1/19	5.3	25/37	67.6	25/34	73.5

uals have been based on both hysterosalpingographic abnormalities and endoscopic abnormalities. Attention has been drawn to the significant false negative and false positive findings when hysterosalpingography alone has been used for the diagnosis of inflammatory residues. The false negative diagnosis produced by radiologic methods in the presence of periadnexal adhesions is significant.<sup>12</sup> Because of this fact all patients not having direct visualization of the pelvic organs were excluded in the current study.

Our studies have confirmed the work of many others. Patients using intrauterine devices have a higher prevalence of inflammatory residues than do nonusers. They also have a higher prevalence of chlamydial antibodies. This same finding has been demonstrated by Moore et al.<sup>12</sup> The increase in residues, however, is only an apparent one. If one examines residue frequency in user versus nonuser groups at the same chlamydial antibody titer level, no difference exists. This is also true if patients with known causes for residues (explained) are excluded and the remainder (unexplained or silent) are grouped according to antibody titer.

**Table VI.** Prevalence of ectopic pregnancy in intrauterine device users and nonusers by titer groups

	Titer group					
	I		II		III	
	n	%	n	%	n	%
Users						
No residues	1/15	6.7	0/5	0	0/0	0
Residues	0/5	0	2/19	10.5	9/25	36
Nonusers						
No residues	3/74	4.1	0/12	0	0/0	0
Residues	2/19	10.5	4/37	10.8	5/34	14.7

These findings suggest that residues found in intrauterine device users are the result of prior chlamydial infection and not the result of the intrauterine device per se.

The increased frequency of chlamydial salpingitis in users may be a function of either increased exposure rate or increased attack rate.<sup>12</sup> The intrauterine device may increase the prevalence of upper tract disease after chlamydial exposure (increased attack rate) by modification of host defenses. On the other hand intrauterine device users may be exposed to *Chlamydia trachomatis* and other sexually transmitted diseases with greater frequency than nonusers (increased exposure rate). The number of prior sexual partners was not a question addressed in our patient interview; however, a correlation between sexually transmitted disease, sexual promiscuity, and pelvic inflammatory disease has been documented by Westrom.<sup>2</sup> Cramer et al.<sup>4</sup> were unable to demonstrate increased tubal infertility in nulliparous

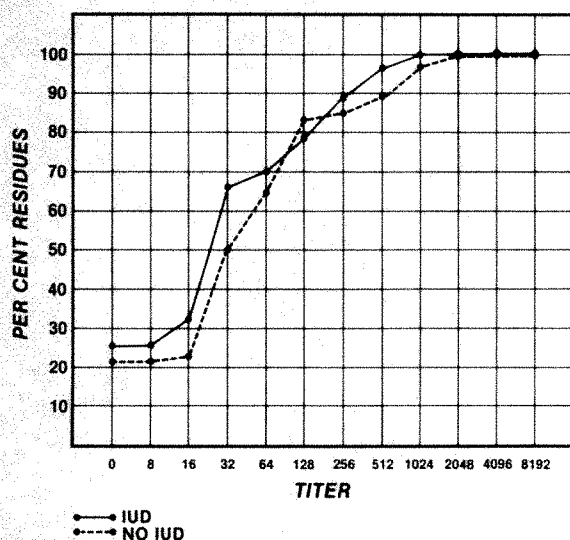


Fig. 1. Graph depicting a running average of three consecutive titer levels from negative to 8192. The corresponding percent prevalence of residues at each titer level in intrauterine device users and nonusers is illustrated. The association between residues and positive titers was highly significant ( $p = .0001$ ).

Of the 245 patients, 144 had a cervical chlamydial culture, 51 had a fluorescein-labeled monoclonal antibody cervical smear (MicroTrak), seven had both a smear and a culture, and 43 (18%) had neither a smear nor a culture performed. Six (4%) of the 151 total cultures were positive. Four (6.9%) of the 58 total smears were positive. One patient in group I had a positive culture, and two had positive smears. Three patients in group II had positive cultures, and one had a positive smear. Two patients in group III had positive cultures, and one had a positive smear. One patient in group II who had both a culture and a smear studied had a positive smear but a negative culture.

Explained causes of adnexal adhesions and tubal occlusion were listed in Material and methods. Table IV illustrates the prevalence of explained causes of adnexal adhesions and tubal occlusions in intrauterine device users and nonusers. Nineteen of 69 (27.6%) users were excluded by means of the criteria for known causes; 39 (22.2%) of 176 nonusers were excluded.

Table V illustrates the prevalence of unexplained ("silent") residues in users and nonusers by titer groups. In group 1 one of 19 nonusers and none of five users had unexplained residues. One patient in this group had bilateral multiple point tubal occlusion and bilateral periovarian and peritubal adhesions but had a negative chlamydial antibody titer and had not used an intrauterine device. She, however, had a positive chlamydial cervical culture. In group 2, 25 of 37 nonusers and 11 of 19 users had unexplained residues, while in group 3, 25 of 34 nonusers and 19 of 25 users had unexplained residues. No significant difference in the

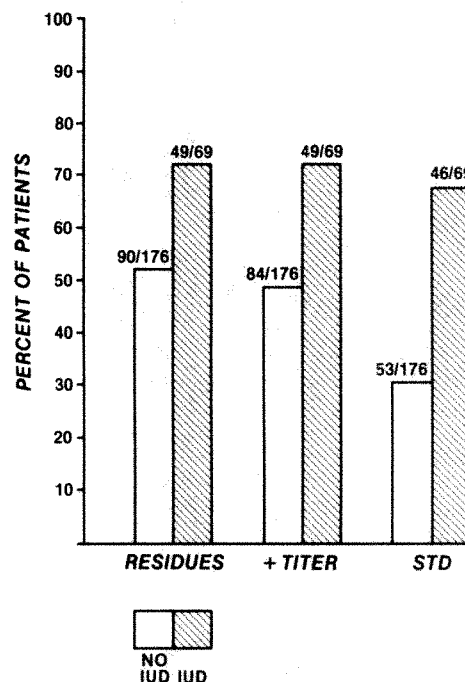


Fig. 2. Graph comparing the prevalence of residues of pelvic inflammatory disease, positive chlamydial antibody titer, and history of sexually transmitted disease in intrauterine device users and nonusers.

prevalence of residues was seen in nonuser versus user groups in any of the three categories.

The greater overall frequency of residues in intrauterine device users versus nonusers parallels the increased frequency both of positive titers and a history of sexually transmitted disease in this population. Forty-nine of 69 (71%) users had a positive titer, while 84 of 176 (47.7%) nonusers had a positive titer. Similarly, 46 of 69 (66.7%) users had a history of sexually transmitted disease and 53 of 176 (30.1%) nonusers had a history of sexually transmitted disease (Fig. 2).

No differences in either bilaterality or severity of residues were seen in users versus nonusers at the same titer levels. No association could be made with a particular type of intrauterine device and frequency, severity, and bilaterality of residues though the numbers are small for statistical validity (Fig. 3). Ectopic pregnancies occurred with greater frequency in patients who had residues of prior infection. The frequency was approximately the same in the negative and low-titer patients with or without intrauterine device use. In the high-titer user group an increased frequency of ectopic pregnancy was noted. However, the number of patients in user and nonuser categories was too small for statistical significance (Table VI).

### Comment

Earlier reports on the relationship of intrauterine device use to tubal infertility and inflammatory resid-



**Table II.** Prevalence of residues in intrauterine device users and nonusers at various ages

Age (yr)	Users		Nonusers	
	n	%	n	%
>35	13/22	59.1	31/51	59.6
30-35	31/41	75.6	36/81	44.4
<30	5/6	83.3	23/44	52.3

(3) documented history of ruptured appendix and peritonitis; (4) documented history of acute gonorrheal or tuberculous salpingitis; (5) documented acute nongonococcal salpingitis; (6) prior ovarian operation with ovarian adhesions only, and tubes normal; (7) endosalpingiosis (histologically proven) with cornual occlusion, tubes normal, and no adhesions.

Sexually transmitted disease was defined as gonorrhea, chlamydia, herpes, condyloma acuminata, *Gardnerella vaginalis*, trichomonas, and mycoplasma-ureaplasma infections. A patient was considered to have had acute gonorrheal salpingitis if she had a positive gonorrheal cervical culture plus clinical symptoms, signs, and confirmatory laboratory findings of salpingitis requiring antibiotic therapy. She was considered to have had acute nongonococcal salpingitis if the signs, symptoms, and laboratory findings were indicative of acute salpingitis but gonorrheal cultures were negative. A patient who had positive gonorrheal and/or chlamydial cervical cultures but who was totally asymptomatic was considered gonorrhea positive or chlamydia positive but was not considered as having had salpingitis.

Continuity corrected  $\chi^2$  was the statistical method used. Statistical analysis of data was performed by Mr. Art Owen, Stanford University, Department of Statistics, Stanford, California.

## Results

Of 245 patients, 176 patients were intrauterine device nonusers and 69 were intrauterine device users. Group I (negative titer) was composed of 93 nonusers and 20 users. Group II (titer of 8 through 256) had 49 nonusers and 24 users, while group 3 (titer of  $\geq 512$ ) had 34 nonusers and 25 users (Table I).

Parity was similar in users and nonusers. Fifteen of 69 (21.7%) users were parous, and thirty-four of 176 (19.3%) nonusers were parous. Users had induced abortions more frequently than had nonusers. Twenty-five of 69 (36.2%) users had induced abortions, and 35 of 176 (19.9%) nonusers had elective pregnancy terminations.

Users tended to be older than nonusers. Only six of 69 (8.7%) users were under 30 years of age, while 44 of 176 (25%) nonusers were younger than 30 years.

**Table III.** Marital status of intrauterine device users and nonusers

Marital status	Users		Nonusers	
	n	%	n	%
Single	3/69	4.3	5/176	2.8
Married one time	39/69	56.5	126/176	71.6
Married two or more times	25/69	36.2	39/176	22.2
Divorced	2/69	2.9	2/176	1.1
Unknown	0/69	0	4/176	2.3

**Table IV.** Prevalence of explained causes of tubal occlusion and/or adnexal adhesions in intrauterine device users and nonusers

Cause	Users	Nonusers
Congenital anomaly	0	1
Endometriosis	0	3
Ruptured appendix	0	2
Gonorrheal salpingitis	6	4
Tuberculous salpingitis	0	2
Nongonorrheal salpingitis	9	19
Adnexal operation	3	8
Endosalpingiosis	1	0

Users had residues with greater frequency at younger ages when compared to nonusers (Table II). Users had two or more marriages more frequently than nonusers, though this is of borderline significance ( $p = 0.05$ ) (Table III).

Overall, 90 of 176 (51.1%) nonusers had residues while 49 of 69 (71.0%) users had residues, indicating a significantly higher prevalence of residues in intrauterine device users ( $p = 0.006$ ). However, when patients were grouped according to level of antibody titer, no significant difference was seen in residue prevalence in nonuser versus user populations ( $p = 0.9$ ). In group I 19 of 93 (20.4%) nonusers had residues and 5 of 20 (25.0%) users had residues ( $p = 0.9$ ). In group II 37 of 49 (75.5%) nonusers had residues and 19 of 24 (79.2%) users had residues ( $p = 0.95$ ). In group III all patients in both nonuser and user categories had residues (Table I).

Although the grouping into categories II and III was an arbitrary one, there was a progressive increase in residue frequency as antibody titer levels rose. The association between antibody titer level and residues was highly significant ( $p = 0.0001$ ). Fig. 1 illustrates a running average of three consecutive titer levels from negative to 8192, with corresponding percentage increases in residue frequency in both users and nonusers. At titers exceeding 512 almost all patients, both users and nonusers, can be expected to have residues. The incidence of residues at any given titer level was not significantly different in nonusers compared to users.

**Table I.** Prevalence of residues in intrauterine device users and nonusers by titer group

	Titer group		
	I	II	III
Users			
No. in group	20	24	25
No residues	15 (75.0%)	5 (20.8%)	0
Residues	5 (25%)	19 (79.2%)	25
Nonusers			
No. in groups	93	49	34
No residues	74 (78.6%)	12 (24.5%)	0
Residues	19 (20.4%)	37 (75.5%)	34

the association between *Chlamydia trachomatis*, sexually transmitted disease, intrauterine device use, and tuboovarian inflammatory residuals.

#### Material and methods

In the years 1983 to 1985 245 patients presented to our office primarily with complaints of infertility. All patients had chlamydial antibody determinations performed at their initial visit and subsequently had direct visualization of pelvic organs by either laparoscopy or laparotomy or both. They form the database for the discussion that follows. In addition, the majority had either a cervical chlamydial culture or a fluorescein-labeled monoclonal antibody smear (MicroTrak, Syva Company).

All patients were interviewed to obtain the following information: marital status, date of birth, history of pelvic inflammatory disease (occurring either before or after intrauterine device use), intrauterine device use (type of device, date used), pelvic infection concurrent with intrauterine device use, sexually transmitted disease, cervical dysplasia, prior pelvic surgery, and prior pregnancy history. In addition, at the time of laparoscopy or laparotomy, adnexal structures were evaluated for presence of tuboovarian adhesions, tubal patency, fimbrial occlusion or phimosis, and agglutination and thickening of fimbrial processes. Type of ovarian adhesions, either capsular or binding, percent of ovarian surface involved by adhesions, and presence of coexisting disease were also noted.

If uncertainty existed regarding type of intrauterine device used, records were obtained relative to the insertion. Only two patients had devices inserted other than the one stated—both historically had used the Dalkon Shield, but a review of records showed that a Cu-7 was inserted in each. Type of device was not available in one patient.

To be included in the study, a patient was required to have had an IgG chlamydial antibody titer and must have had direct visualization of her pelvic organs.

Antibody testing was performed by Virolabs in Berkeley, California, by an indirect fluorescent antibody test on an L2 serovar of *Chlamydia trachomatis*. With this method an antibody titer of 1:8 is considered positive. Cultures were performed by a standard tissue culture technique with use of cycloheximide-treated McCoy cells on 12 mm cover slips. Cytologic evaluation of monoclonal antibody stained smears was performed by the MicroTrak kit marked by Syva Company for that purpose.

Patients were grouped into three categories. Group I consisted of patients with negative chlamydial IgG antibody titers. Group II was composed of patients with positive titers ranging from 1:8 through 1:256, and group III from 1:512 or greater. All three categories were then subgrouped into those patients who had not used intrauterine devices (nonusers) and those who had used intrauterine devices (users). The six groups were then assessed for evidence of inflammatory residuals and coexisting diseases at time of visualization of the pelvis. They were also assessed for a history of sexually transmitted diseases, pelvic infection before or after intrauterine device usage, pelvic infection with the intrauterine device in place, prior ectopic pregnancies, and prior pelvic operations.

"Residues of pelvic inflammatory disease" includes a spectrum of post infectious pelvic abnormalities ranging from severe periadnexal adhesions and tubal occlusion to mild adhesions and minimal tubal epithelial damage. It is sometimes difficult to assign a specific etiologic role to infection when coexisting diseases are present. Adhesions and even tubal occlusion may result from prior pelvic operations, endometriosis, pelvic peritonitis secondary to ruptured appendix, and other conditions in the absence of pelvic inflammatory disease. Intrauterine device users and nonusers may develop acute, overt salpingitis, after which they can be expected to develop laparoscopically demonstrable residues with significant frequency. In this study all patients who had laparoscopy and a chlamydial antibody titer performed were included in the database irrespective of the presence of coexisting diseases or a history of clinically documented acute salpingitis. Since both "intrauterine device-related" salpingitis and chlamydial salpingitis are typically "silent" infections, the database was reexamined, and those patients who had specific probable causes for tubal occlusion and periadnexal adhesions were excluded (listed in next paragraph). The remaining patients were then assessed for residue frequency (unexplained or silent) in nonusers and users in the three titer groups.

Explained causes of tubal occlusion and adnexal adhesions are as follows: (1) congenital tubal abnormality resulting in ampullary occlusion; (2) endometriosis with ovarian adhesions only, and tubes normal;

# Residues of pelvic inflammatory disease in intrauterine device users: A result of the intrauterine device or *Chlamydia trachomatis* infection?

A. Maynard Guderian, M.D., and Gerald E. Trobough, M.D.

Los Gatos, California

It is currently believed that intrauterine devices cause pelvic inflammatory disease and tubal infertility. To investigate this concept further, we evaluated 245 infertile patients for inflammatory residues by laparoscopy or laparotomy; 176 patients had not used an intrauterine device and 69 had used one. Chlamydial antibody titers were performed on all patients. Although users had a higher overall prevalence of inflammatory residues than nonusers, there was no difference in residue prevalence for either group at the same titer level. No specific type of device appeared to be associated with either an increased or decreased residue frequency. "Silent" chlamydial infections occurred with equal frequency in both users and nonusers. We conclude that inflammatory residues and tubal infertility in intrauterine device users are not caused by the intrauterine device but by both overt and silent chlamydial infections. (AM J OBSTET GYNECOL 1986;154:497-503.)

**Key words:** Pelvic inflammatory disease, intrauterine device, chlamydia, chlamydial antibody titer, tubal infertility

Numerous publications in the past 15 years have established a significant association between intrauterine device usage and acute pelvic inflammatory disease<sup>1</sup> resulting in postinflammatory pelvic adhesions and tubal damage. These factors are important in the development of tubal infertility<sup>2,3</sup> and ectopic pregnancy. Although epidemiologic studies have shown a relative risk of pelvic inflammatory disease in intrauterine device users of 2.5 to 7.3 times the risk of nonusers of contraception,<sup>1</sup> only recently have reports established a relationship between intrauterine device use and tubal infertility.<sup>4,5</sup>

Although there may be an increased risk of gonorrheal salpingitis in intrauterine device users compared to nonusers, the preponderance of intrauterine device infections appears to be of nongonococcal origin. The clinical characteristics of nongonococcal pelvic inflammatory disease are typically either subclinical with residuals discovered only during infertility investigation,<sup>6</sup> or if symptoms are present, a protracted, indolent course characterized by little or no fever, abnormal uterine bleeding, mild pain, and elevated sedimentation rate.<sup>7</sup> There appears to be

a correlation between the incidence of acute salpingitis and sexually transmitted diseases and the number of sexual partners.<sup>8,9</sup>

Since 1977 increasing numbers of reports of acute salpingitis caused by *Chlamydia trachomatis* have been published, first by European<sup>10</sup> and more recently by American investigators.<sup>11,12</sup> That chlamydia can produce endometritis and salpingitis has been experimentally documented in grivet monkeys and pig-tailed monkeys. The clinical course of human acute chlamydial salpingitis is remarkably similar to that described above for nongonococcal intrauterine device-related infections. At laparoscopy a greater degree of tubal damage can be demonstrated than would be anticipated from the rather minimal clinical symptoms and signs.<sup>7</sup>

Subclinical chronic chlamydial salpingitis has been theorized but has been documented only recently by Henri-Suchet et al.<sup>13</sup> in 1981 and Shepard et al.<sup>14</sup> in 1985. However, prior and current chlamydial infection can be demonstrated by use of an IgG microimmunofluorescent antibody technique, and this method has been used by several American groups to study the association between chlamydia and subsequent tubal infertility.<sup>11,12,15,16</sup> Gump et al.<sup>16</sup> state that both intrauterine device use and positive chlamydial antibody appear to be positively correlated with inflammatory residuals and make the suggestion that both are independent variables.

It is the purpose of this report to investigate further

From the Los Olivos Womens Medical Clinic, Inc., and Los Olivos Medical Research Foundation, Inc.

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# A Standard in Progesterone Therapy

## Provera<sup>®</sup> 10 MG TABLETS (medroxyprogesterone acetate)

### WARNING

THE USE OF PROGESTATIONAL AGENTS DURING THE FIRST FOUR MONTHS OF PREGNANCY IS NOT RECOMMENDED.

Progestational agents have been used beginning with the first trimester of pregnancy in an attempt to prevent habitual abortion or treat threatened abortion. There is no adequate evidence that such use is effective and there is evidence of potential harm to the fetus when such drugs are given during the first four months of pregnancy. Furthermore, in the vast majority of women, the cause of abortion is a defective ovum, which progestational agents could not be expected to influence. In addition, the use of progestational agents, with their uterine-relaxant properties, in patients with fertilized defective ova may cause a delay in spontaneous abortion. Therefore, the use of such drugs during the first four months of pregnancy is not recommended.

Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies including congenital heart defects and limb reduction defects. One study estimated a 4.7-fold increased risk of limb reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 in 1000.

If the patient is exposed to PROVERA Tablets (medroxyprogesterone acetate) during the first four months of pregnancy or if she becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus.

**INDICATIONS:** Secondary amenorrhea; abnormal uterine bleeding due to hormonal imbalance in the absence of organic pathology such as fibroids or uterine cancer.

**CONTRAINDICATIONS:** Thrombophlebitis, thromboembolic disorders, cerebral apoplexy or patients with a past history of these conditions. Liver dysfunction or disease. Known or suspected malignancy of breast or genital organs. Undiagnosed vaginal bleeding. Missed abortion. Known sensitivity to medroxyprogesterone acetate. As a diagnostic test to pregnancy.

**WARNINGS:** 1. Immediately discontinue administration should any of the following thrombotic disorders occur or be suspected: thrombophlebitis, cerebrovascular disorders, pulmonary embolism, retinal thrombosis. 2. Beagle dogs treated with medroxyprogesterone acetate developed mammary nodules some of which were malignant. Although nodules occasionally appeared in control animals, they were intermittent in nature; whereas the nodules in the drug treated animals were larger, more numerous, persistent, and there were some breast malignancies with metastases. Their significance with respect to humans has not been established. 3. Discontinue medication pending examination if there is sudden partial or complete loss of vision, onset of proptosis, diplopia, or migraine. If papilledema or retinal vascular lesions occur, withdraw medication. 4. Detectable amounts of progestin have been identified in the milk of mothers receiving the drug. The effect of this on the nursing infant has not been determined. 5. Usage in pregnancy is not recommended (see Warning box). 6. Three major studies in Great Britain and one in this country have shown a statistically significant association between thrombophlebitis, pulmonary embolism, cerebral thrombosis and embolism and the use of oral contraceptives. It has been estimated that users are several times as likely to undergo thromboembolic disease without evident cause as nonusers. The American study indicated that the risk did not persist after discontinuation, and it was not enhanced by long-continued administration.

**PRECAUTIONS:** A pretreatment physical exam should include special reference to breast and pelvic organs and a Papanicolaou smear. This drug may cause fluid retention; therefore, carefully observe patients with conditions influ-

enced by fluid retention such as epilepsy, migraine, asthma, and cardiac or renal dysfunction. In irregular bleeding per vaginam, bear in mind nonfunctional causes and perform adequate diagnostic measures. Advise pathologist of therapy when submitting relevant specimens. Carefully observe patients with history of psychic depression and discontinue drug if serious depression recurs. Any possible influence of prolonged therapy on pituitary, ovarian, adrenal, hepatic, or uterine function awaits further study. Decreased glucose tolerance has been observed in a small percentage of patients on estrogen-progestin combinations; therefore, carefully observe diabetic patients receiving progestin therapy. Age constitutes no absolute limiting factor, although onset of climacteric may be masked. Because of the occasional occurrence of thrombotic disorders (thrombophlebitis, pulmonary embolism, retinal thrombosis, and cerebrovascular disorders) in patients taking estrogen-progestin combinations and since the mechanism is obscure, the physician should be alert to the earliest manifestation of these disorders. (See Patient Information for complete prescribing information.)

**ADVERSE REACTIONS: Pregnancy:** (see Warning box); **Breast:** rare reports of breast tenderness or galactorrhea; **Skin:** sensitivity reactions including pruritus, urticaria, edema and generalized rash; acne, alopecia and hirsutism in a few patients; **Thromboembolic Phenomena** including thrombophlebitis and pulmonary embolism.

The following adverse reactions have been observed in women taking progestins including medroxyprogesterone acetate: breakthrough bleeding; spotting; change in menstrual flow; amenorrhea; edema; change in weight; changes in cervical erosion and secretions; cholestatic jaundice; rash (allergic) with and without pruritus; mental depression; anaphylaxis and anaphylactoid reactions; pyrexia; insomnia; nausea and somnolence.

A statistically significant association has been demonstrated between use of estrogen-progestin combination drugs and the serious adverse reactions of thrombophlebitis, pulmonary embolism and cerebral thrombosis and embolism. Therefore, patients on progestin therapy should be carefully observed.

Although available evidence is suggestive, a relationship has been neither confirmed nor refuted for the association of the serious adverse reaction of neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been observed in patients receiving estrogen-progestin combination drugs: rise in blood pressure in susceptible individuals; premenstrual-like syndrome; changes in libido; changes in appetite; cystitis-like syndrome; headache; nervousness; dizziness; fatigue; backache; hirsutism; loss of scalp hair; erythema multiforme; erythema nodosum; hemorrhagic eruption; and itching. Therefore, observe patients on progestin therapy carefully.

The following laboratory results may be altered by the use of estrogen-progestin combination drugs: increased sulfobromophthalein retention and other hepatic function tests; coagulation tests (increase in prothrombin factors VII, VIII, IX and X); metyrapone test; pregnanediol determination; thyroid function tests (increase in PBI, and butanol extractable protein bound iodine and decrease in T<sub>3</sub> uptake values).

**Caution:** Federal law prohibits dispensing without prescription.

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# A Standard



**Provera**<sup>®</sup> 10 MG  
TABLETS  
(medroxyprogesterone acetate)

**A Standard in Progesterone Therapy**

Please see adjacent page for brief summary of prescribing information.



# Mefoxin<sup>®</sup> IV<sub>IM</sub> (Cefoxitin Sodium | MSD)

**Indications and Usage:** *Treatment*—Serious infections caused by susceptible strains of the designated microorganisms in the following diseases:

**LOWER RESPIRATORY TRACT INFECTIONS**, including pneumonia and lung abscess, caused by *Streptococcus pneumoniae* (formerly *Diplococcus pneumoniae*), other streptococci (excluding enterococci, e.g., *Strep. faecalis*), *Staphylococcus aureus* (penicillinase and non-penicillinase producing), *Escherichia coli*, *Klebsiella* species, *Hemophilus influenzae*, and *Bacteroides* species.

**GENITOURINARY INFECTIONS**, urinary tract infections caused by *E. coli*, *Klebsiella* species, *Proteus mirabilis*, indole-positive *Proteus* (i.e., *P. morganii*, *P. rettgeri*, and *P. vulgaris*), and *Providencia* species. Uncomplicated gonorrhea due to *Neisseria gonorrhoeae* (penicillinase and non-penicillinase producing).

**INTRA-ABDOMINAL INFECTIONS**, including peritonitis and intra-abdominal abscess, caused by *E. coli*, *Klebsiella* species, *Bacteroides* species including the *B. fragilis* group, and *Clostridium* species.

**GYNCOLOGICAL INFECTIONS**, including endometritis, pelvic cellulitis, and pelvic inflammatory disease, caused by *E. coli*, *N. gonorrhoeae* (penicillinase and non-penicillinase producing), *Bacteroides* species including the *B. fragilis* group, *Clostridium* species, *Peptococcus* species, *Peptostreptococcus* species, and group B streptococci.

**SEPTICEMIA** caused by *Strep. pneumoniae* (formerly *D. pneumoniae*), *Staph. aureus* (penicillinase and non-penicillinase producing), *E. coli*, *Klebsiella* species, and *Bacteroides* species including the *B. fragilis* group.

**BONE AND JOINT INFECTIONS** caused by *Staph. aureus* (penicillinase and non-penicillinase producing).

**SKIN AND SKIN-STRUCTURE INFECTIONS** caused by *Staph. aureus* (penicillinase and non-penicillinase producing), *Staph. epidermidis*, streptococci (excluding enterococci, e.g., *Strep. faecalis*), *E. coli*, *P. mirabilis*, *Klebsiella* species, *Bacteroides* species including the *B. fragilis* group, *Clostridium* species, *Peptococcus* species, and *Peptostreptococcus* species.

Although appropriate culture and susceptibility studies should be performed, therapy may be started while awaiting these results. Cefoxitin is not active *in vitro* against most strains of *Pseudomonas aeruginosa* and enterococci (e.g., *Strep. faecalis*) and many strains of *Enterobacter cloacae*. Methicillin-resistant staphylococci are almost uniformly resistant to cefoxitin.

**Contraindications:** Previous hypersensitivity to cefoxitin and the cephalosporin group of antibiotics.

**Warnings:** BEFORE THERAPY IS INSTITUTED, CAREFUL INQUIRY SHOULD BE MADE TO DETERMINE PREVIOUS HYPERSENSITIVITY REACTIONS TO CEFOTILIN, CEPHALOSPORINS, PENICILLINS, OR OTHER DRUGS. GIVE WITH CAUTION TO PENICILLIN-SENSITIVE PATIENTS. ANTIBIOTICS SHOULD BE ADMINISTERED WITH CAUTION TO ANY PATIENT WHO HAS DEMONSTRATED SOME FORM OF ALLERGY PARTICULARLY TO DRUGS. IF AN ALLERGIC REACTION TO CEFOTILIN OCCURS, DISCONTINUE THE DRUG. SERIOUS HYPERSENSITIVITY REACTIONS MAY REQUIRE EPINEPHRINE AND OTHER EMERGENCY MEASURES.

**Pseudomembranous colitis**, from mild to life-threatening in severity, has been reported with virtually all antibiotics (including cephalosporins); therefore, it is important to consider its diagnosis when diarrhea develops in association with antibiotic use. Broad-spectrum antibiotics alter normal flora of colon and may permit overgrowth of clostridia; a toxin produced by *Clostridium difficile* is a primary cause of antibiotic-associated colitis. Mild cases may respond to drug discontinuance alone; in more severe cases, management may include sigmoidoscopy, appropriate bacteriological studies, fluid, electrolyte and protein supplementation, and use of a drug such as oral vancomycin; isolation of the patient may be advisable. Other causes of colitis should also be considered.

**Precautions:** *General*—Total daily dose should be reduced in patients with reduced urinary output due to renal insufficiency because high and prolonged serum antibiotic concentrations can occur from usual doses. Prescribe with caution in patients with a history of gastrointestinal disease, particularly colitis. Prolonged use may result in overgrowth of nonsusceptible organisms; repeated evaluation of the patient's condition is essential. If superinfection occurs, take appropriate measures.

*Drug Interactions*—Increased nephrotoxicity has been reported following concomitant administration of cephalosporins and aminoglycoside antibiotics.

*Drug/Laboratory Test Interactions*—High concentrations (>100 mcg/mL) may interfere with measurement of serum and urine creatinine levels by the Jaffe reaction and produce false increases of modest degree in creatinine levels reported; serum samples should not be analyzed for creatinine if withdrawn within 2 hours of cefoxitin administration. High concentrations may interfere with measurement of urinary 17-hydroxy-corticosteroids by the Porter-Silber reaction and produce false increases of modest degree in levels reported. A false-positive reaction for glucose in urine has been observed with CLINITEST<sup>®</sup> reagent tablets.

*Carcinogenesis, Mutagenesis, Fertility Impairment*—No long-term animal study has been performed on carcinogenic or mutagenic potential. Rat studies at approximately three times maximum recommended human dosage revealed no effects on fertility or mating ability.

*Pregnancy Category B*—Reproduction studies in rats and mice did not reveal teratogenic or fetal toxic effects, although fetal weights were slightly decreased. In rabbits, cefoxitin was associated with a high incidence of abortion and maternal death, neither considered teratogenic. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

*Nursing Mothers*—Excreted in human milk. Exercise caution.

*Pediatric Use*—Safety and efficacy in infants from birth to three months have not yet been established. In children three months and older, higher doses have been associated with increased incidence of eosinophilia and elevated SGOT.

**Adverse Reactions:** The most common adverse reactions have been local reactions following intravenous or intramuscular injection. Other adverse reactions have been encountered infrequently. *Local Reactions*—Thrombophlebitis with intravenous administration; pain, induration, and tenderness after intramuscular injections. *Allergic Reactions*—Rash (including exfoliative dermatitis), pruritus, eosinophilia, fever, and other allergic reactions including anaphylaxis. *Cardiovascular*—Hypotension. *Gastrointestinal*—Diarrhea, including documented pseudomembranous colitis during or after treatment, and, rarely, nausea and vomiting. *Blood*—Eosinophilia, leukopenia including granulocytopenia, neutropenia, anemia, including hemolytic anemia, thrombocytopenia, and bone marrow depression. A positive direct Coombs test may develop in some individuals, especially those with azotemia. *Liver Function*—Transient elevations in SGOT, SGPT, serum LDH, and serum alkaline phosphatase. *Renal Function*—Elevations in serum creatinine and/or blood urea nitrogen levels and, rarely, acute renal failure.

**Note:** In group A beta-hemolytic streptococcal infections, therapy should be maintained for at least 10 days to guard against the risk of rheumatic fever or glomerulonephritis. In staphylococcal and other infections involving a collection of pus, surgical drainage should be carried out where indicated. Intramuscular injections should be well within the body of a relatively large muscle such as the upper outer quadrant of the buttock (i.e., gluteus maximus); aspiration is necessary to avoid inadvertent injection into a blood vessel. The total daily dosage in infants and children should not exceed 12 grams.

**How Supplied:** Sterile cefoxitin sodium in vials and infusion bottles containing 1 gram or 2 grams cefoxitin equivalent and in 10-gram bulk bottles.

<sup>1</sup> *B. fragilis*, *B. distasonis*, *B. ovalis*, *B. theta*, *Haemophilus*, *B. vulgaris*.

<sup>2</sup> Registered trademark of Ames Company, Division of Miles Laboratories, Inc.

For more detailed information, consult your MSD Representative or see Prescribing Information, Merck Sharp & Dohme, Division of Merck & Co., Inc., West Point, PA 19486.

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**fact: documented clinical efficacy**

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---

**fact: safety comparable to cephalothin**

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---

Pseudomembranous colitis, from mild to life-threatening in severity, has been reported with virtually all antibiotics (including cephalosporins); therefore, it is important to consider its diagnosis when diarrhea develops in association with antibiotic use.

†MEFOXIN is indicated for the treatment of pelvic inflammatory disease caused by *Escherichia coli*, *Neisseria gonorrhoeae* (penicillinase- and non-penicillinase-producing), *Bacteroides* species including the *Bacteroides fragilis* group, *Clostridium* species, *Peptococcus* species, *Peptostreptococcus* species, and Group B streptococci. MEFOXIN is not indicated for treatment of pelvic inflammatory disease where *Chlamydia trachomatis* is present or suspected.

\*proven for treatment of, and prophylaxis against, infections due to indicated organisms

\*\**In vitro* activity does not necessarily imply *in vivo* effectiveness.

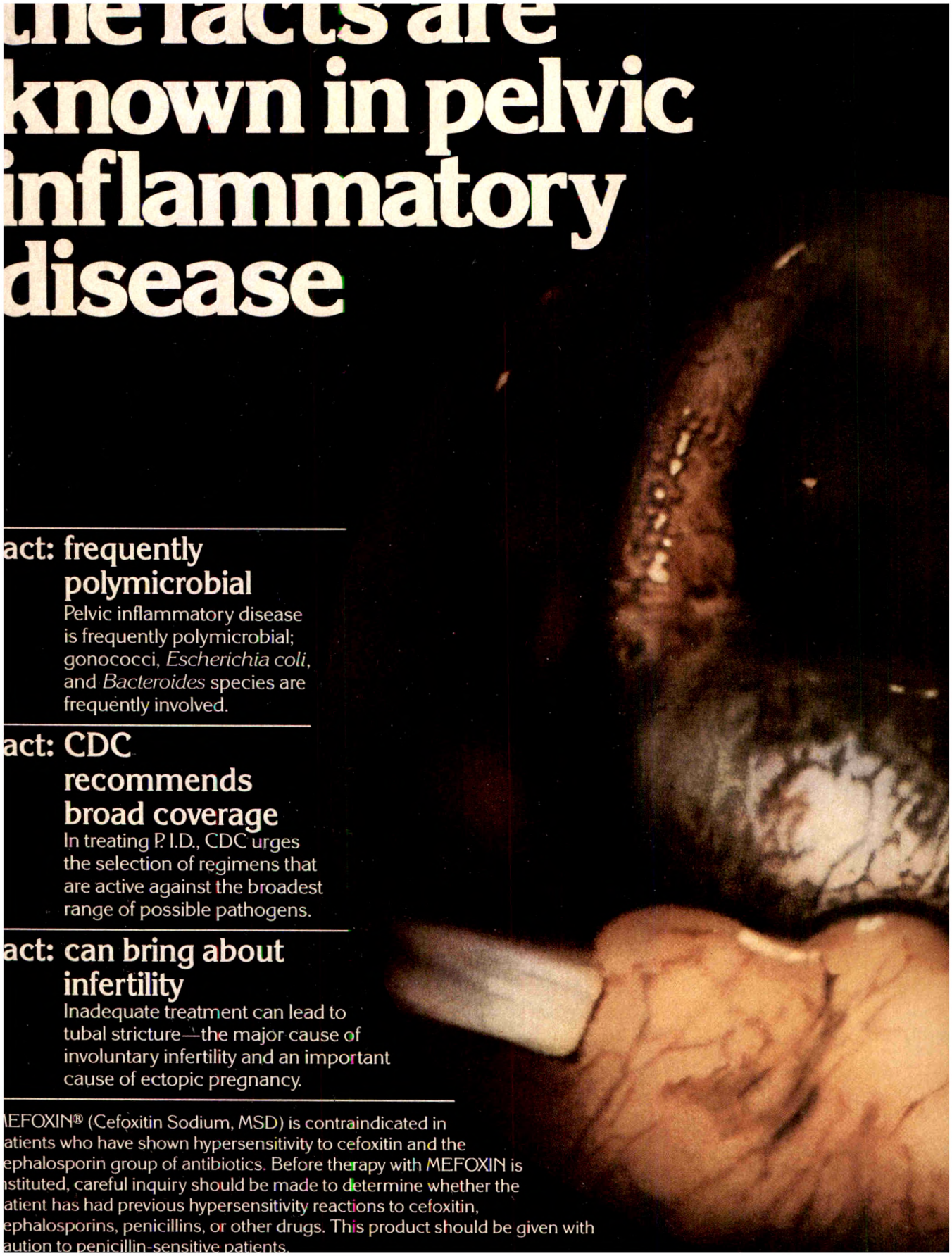
For a Brief Summary of Prescribing Information, please see following page.

**MSD**  
MERCK  
SHARP  
&  
DOHME

Unruptured ampullary  
ectopic pregnancy.



# The facts are known in pelvic inflammatory disease



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## Fact: frequently polymicrobial

Pelvic inflammatory disease is frequently polymicrobial; gonococci, *Escherichia coli*, and *Bacteroides* species are frequently involved.

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## Fact: CDC recommends broad coverage

In treating P.I.D., CDC urges the selection of regimens that are active against the broadest range of possible pathogens.

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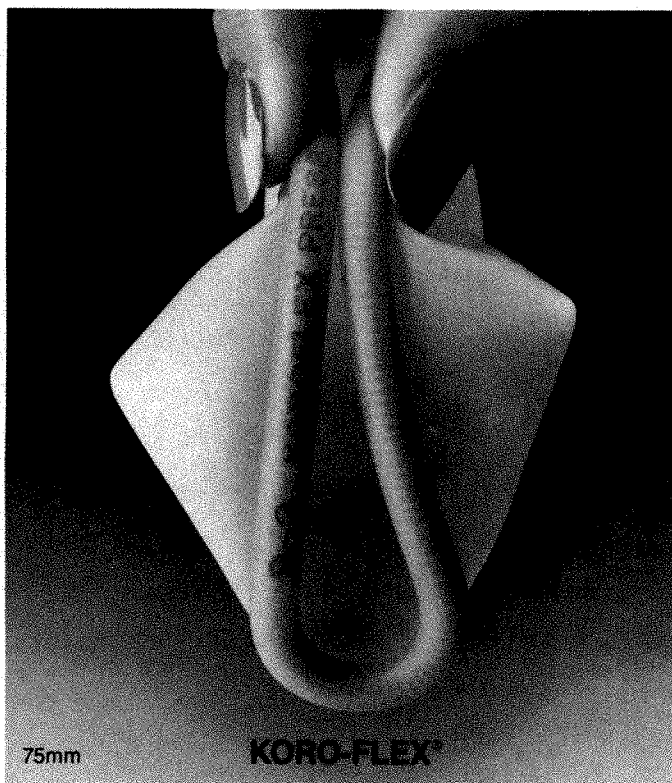
Inadequate treatment can lead to tubal stricture—the major cause of involuntary infertility and an important cause of ectopic pregnancy.

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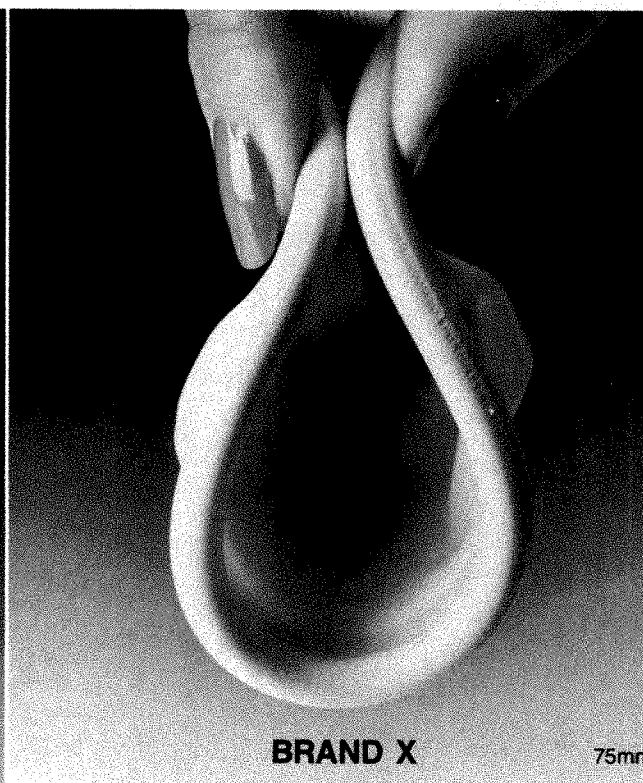
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**Fig. 3.** Condyloma of the distal penile shaft. (Original magnification  $\times 9$ .)

quently found to have evidence of condyloma on slide review. Thus 37 of 45 patients had condyloma not visible to the naked eye.

Table I depicts the histologic diagnosis of the eight men who were originally thought to have negative biopsy specimens. On review of the biopsy specimens, one man demonstrated features of condyloma; a second man underwent repeat colposcopic examination and demonstrated evidence of condyloma. Two of the six patients who did not demonstrate condyloma on the biopsy specimen had other dermatologic lesions of the penis. Of the four remaining negative biopsy specimens, two demonstrated features of chronic inflammation and epithelial thickening that would be expected to provide a "false positive" colposcopic result. Two biopsy specimens demonstrated normal penile epithelium.

Cytologic evidence of abnormal epithelium was found in a total of 13 men, either on direct swab of the meatus or on urine cytologic examination. This experience is depicted in Table II.

**Table III.** Management of condyloma

Women		Men
Papanicolaou smear Colposcopy and directed biopsy		Papanicolaou smear, urine cytologic testing Colposcopy and directed biopsy
Atypical Mitotic Figures Absent		YAG laser
Atypical Mitotic Figures Present		5-Fluorouracil cream
Cryotherapy Topical 5-fluorouracil cream		Carbon dioxide laser
		Podophyllin

Condoms are to be used for 1 year. Reexamination by colposcopy is to be done in 3 months.

### Comment

Murphy et al.<sup>3</sup> have demonstrated evidence of condyloma within the meatus and urethra of male partner of women with dysplasia and condyloma. We have not routinely subjected these men to cystourethroscopic evaluation pending review of these slides. It would seem reasonable to use urine and/or meatal cytologic examination to screen for condyloma and suggest urethroscopic examination for any positive cytologic tests.

Our experience has demonstrated that a very large number of men who are partners of women with condyloma will have histologic evidence of penile condyloma. Contrary to standard medical opinion, these lesions are not visible to the naked eyes. Magnification and acetic acid are required to demonstrate these lesions.

Our current management scheme for couples with condyloma is depicted in Table III. We use the presence or absence of atypical mitotic figures to guide treatment in women. We believe that ample evidence exists that condyloma involves both members of a sexual partnership. Precise details regarding treatment and location remain to be defined. Colposcopy appears to be an invaluable aid in establishing the diagnosis of penile condyloma.

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**Fig. 1.** Penile condyloma involving the corona. (Original magnification  $\times 9$ .)



**Fig. 2.** Penile condyloma of the midshaft. (Original magnification  $\times 9$ .)

**Table I.** Histologic findings in patients without condyloma

Case No.	Finding
1	Chronic inflammation
2	Chronic inflammation
3*	Chronic inflammation
4*	Chronic inflammation
5	Dilated sweat duct
6	Lichen nitidus
7	Verruca vulgaris
8	Penile skin tag

\*Subsequent biopsy specimen revealed condyloma.

elevated acetowhite focal lesions were identified. Local anesthesia was induced with 1% lidocaine without epinephrine via a tuberculin syringe with a 26-gauge needle. Lesions were grasped with a small forceps under colposcopic guidance, elevated, and excised in a superficial fashion with a 15 blade scalpel.

### Results

Fifty-one patients were examined. All demonstrated elevated white penile lesions colposcopically consistent with condyloma. No patients without lesions were seen.

**Table II.** Cytologic and histologic findings

Finding	No.
Abnormal meatus, cytologic finding	7/50
Abnormal urine, cytologic finding	6/32
Biopsy-confirmed condyloma	45/51

The lesions varied from a very small, focal white lesion with subtle epithelial changes to a widespread coarse white epithelium with hyperkeratotic features. The patients evaluated were primarily young men. The average age was 29, with a range of 19 to 40. Representative lesions are demonstrated in Figs. 1, 2, and 3.

Most of the men experienced coitus for the first time during adolescence. Age of first coitus ranged from 11 to 25, with the mean age being 17.5. The number of sexual partners varied from one to 75. The mean was 14.7.

Of the 51 men examined and found to have suspicious lesions, eight were identifiable with the naked eye and confirmed histologically, and 35 histologically confirmed lesions were seen only with colposcopy; two additional men who were originally thought to have histologically negative biopsy specimens were subse-

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## Colposcopy in the diagnosis of penile condyloma

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To determine the incidence of penile condyloma in a group of high-risk men, we carried out colposcopy and biopsy of suspicious lesions in 51 men. All men were partners of women with condyloma. Of these men, 45 were found to have histologic evidence of condyloma, and only eight of these had grossly visible disease. (*AM J OBSTET GYNECOL* 1986;154:494-6.)

**Key words:** Condyloma, colposcopy, penile condyloma

The venereal nature of cervical neoplasia and condyloma has been long established. Specific details relating to the route of transmission, reservoir sites if any, and frequency of penile condyloma have been elusive. Baggish,<sup>1</sup> in 1982, in a discussion of recurrent cervical and vaginal condyloma, noted parenthetically that 82% of male partners of women with recurrent condyloma were found to have penile condyloma. Levine et al.,<sup>2</sup> in 1984, prospectively evaluated a series of male partners of women with cervical dysplasia and found an incidence of penile condyloma in that subset of patients of 64%.

From April 1, 1984, to October 31, 1984, we have attempted to prospectively evaluate the frequency of penile condyloma in the male partners of women referred to the gynecologic oncology section of the De-

partment of Obstetrics and Gynecology at Pennsylvania Hospital for evaluation and treatment of abnormal Papanicolaou smears.

### Material and methods

Patients who demonstrated histologic evidence of condyloma on colposcopically directed biopsy specimens were asked to have their partner(s) come to the gynecologic oncology section for a colposcopic examination of the penis. Each male partner who was examined was asked to provide a urine specimen for cytologic evaluation. A history of previous condylomatous or other penile lesions or other venereal diseases as well as information regarding age of first coitus and number of sexual partners was elicited. A Papanicolaou smear was obtained from the urethral meatus with the use of a Calgi swab moistened with normal saline solution. The penis was inspected grossly for evidence of visible penile lesions. The penis was swabbed with a large proctoscopy swab dipped in 5% acetic acid and 2 to 4 minutes elapsed before colposcopic examination. The epithelial surface of the penis was evaluated colposcopically by means of a Leisegang 3b colposcope with magnification of 7.5 to 30 power. Representative

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herent to the preeclamptic state.<sup>5</sup> The hemodynamic picture of patients in category II appears to represent a response to fluid therapy which unmasks an inherent volume expansion—unresponsive but arteriodilator-responsive renal arteriospasm. Unfortunately, neither clinical assessment nor measurement of central venous pressure alone reliably differentiates these oliguric states. Clearly, blind volume expansion would have been deleterious in at least 2 of these subjects, Patients 8 and 9. Noninvasive assessment of maternal cardiac output and calculation of systemic vascular resistance may prove to be helpful in differentiating these subsets of oliguric patients,<sup>10</sup> but at the present time, management of such patients should be guided with a pulmonary artery catheter. Because most oliguric patients fall into category I, we recommend a 500 ml fluid challenge, with use of lactated Ringer's solution (in addition to expeditious delivery) in the oliguric, severe preeclamptic patient. Should the patient fall into category II or even III, this volume will not have disastrous consequences, yet such a single "blind" fluid challenge will be effective in most cases and will save many patients the risks involved in invasive hemodynamic monitoring. Should such a fluid challenge not prove adequate, further therapy should be guided by a pulmonary artery catheter to avoid the development of pulmonary or cerebral edema.

The use of crystalloid rather than colloid solutions remains controversial in preeclampsia as well as in other areas of critical care medicine. Peripartum colloid osmotic pressure is reduced in patients with preeclampsia.<sup>11</sup> Increasing intravascular colloid osmotic pressure by infusion of a solution such as 5% albumin has theoretical advantages in avoiding pulmonary edema. However, such a theoretical advantage must be weighed against the risks of actually inducing pulmonary edema by increasing extravascular osmotic pressure, should significant pulmonary capillary leakage occur. This phenomenon has not been well studied in the preeclamptic patient, although such a leak has been proven in at least some patients.<sup>12</sup> Thus we use crystalloid exclusively in preeclamptic patients with satisfactory results. However, no data exist to document the benefit of crystalloid rather than colloid infusion in such patients.

Finally, the need for active management of oliguria (other than delivery) in the patient with severe preeclampsia remains controversial. Acute renal failure, resulting from acute tubular or cortical necrosis, is a well-documented complication of severe preeclampsia. Indeed preeclampsia and abruptio placentae are the most common causes of acute renal failure in pregnancy.<sup>13</sup> In most reported cases, acute renal failure has been associated with persistent oliguria<sup>14</sup>; however, in individual cases it is not clear whether oliguria was a

cause of or simply a manifestation of acute renal failure. Under many other conditions it is well established that persistent oliguria secondary to decreased renal perfusion may lead to acute tubular necrosis and that maintenance of urine output is vital in preventing this complication. Severe and prolonged selective renal vasospasm in conjunction with the hypercoagulability of pregnancy has also been implicated in the genesis of acute cortical necrosis.<sup>15</sup> It seems likely that the low incidence of acute tubular necrosis in the oliguric preeclamptic patient is more a function of the significant capacity of the kidneys in young, healthy women to tolerate substantial ischemic insult than of an inherent resistance of the preeclamptic kidney to acute tubular necrosis. In addition, since persistent oliguria itself is an indication for delivery in the preeclamptic patient, prompt removal of the products of conception with prompt resolution of the preeclamptic state may generally be anticipated. The authors have observed two cases of severe preeclampsia in which neglected, persistent oliguria was associated with acute tubular necrosis; one case required chronic dialysis. Certainly this is a very rare occurrence, and the risk of overhydration, with resultant pulmonary or cerebral edema, is very real in such patients.<sup>16</sup> Furthermore, until the present report the hemodynamics associated with the oliguric state in the severe preeclamptic patient have not been described. Thus given the substantial risk of complications resulting from attempts to reverse oliguria with blind volume expansion, a conservative approach of nonintervention (other than delivery) in the oliguric, severe preeclamptic patient may be justified in cases where transfer is not available. However, in perinatal centers where pulmonary artery catheterization is available for the complicated obstetric patient, oliguria may be promptly and safely corrected with use of the management scheme outlined. Such an approach will further reduce the risk of clinical or subclinical renal damage in the severe preeclamptic patient with persistent oliguria. During the antepartum period, beneficial effects on uterine blood flow and the ability of the fetus to tolerate the stress of labor is also an attractive, although unproven, consideration. Additional investigations in this area seem warranted.

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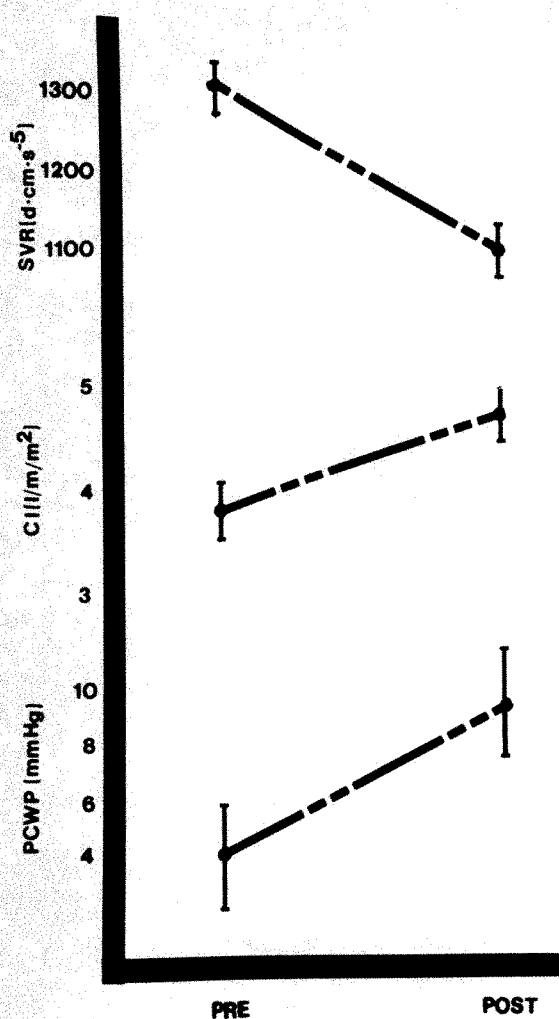


Fig. 1. Hemodynamic changes accompanying volume infusion in five preeclamptic patients in category I. Values represent mean and standard deviations. PCWP = Pulmonary capillary wedge pressure. CI = Cardiac index. SVR = Systemic vascular resistance.

teriospasm. Volume infusion resulted in decreased systemic vascular resistance, elevation of pulmonary capillary wedge pressure and cardiac index, and resolution of the oliguria, without changes in mean arterial pressure. Similar hemodynamic changes with volume expansion were reported by Groenendijk et al.<sup>9</sup> in non-oliguric patients. As this is the typical hemodynamic picture reported in most patients with severe preeclampsia, it is likely that the majority of oliguric patients who respond to fluid challenge without invasive monitoring also fall into this hemodynamic subset.

The hemodynamic profile exhibited by patients 6 to 8 (category two) was similar to that observed in group 1, except that the pulmonary capillary wedge pressure was in the upper range of normal or elevated and the systemic vascular resistance was somewhat lower (Table I). These changes likely reflect the results of volume infusion before pulmonary artery catheterization. In

these patients, intravascular volume should have been adequate to maintain urine output. Under such circumstances, persistent oliguria with concentration of urine, in the presence of essentially normal systemic vascular resistance suggests renal hypoperfusion caused by a selective degree of renal arteriospasm beyond that reflected in measurement of systemic vascular resistance. Such renal arteriospasm appears to have been unresponsive to volume expansion or even overexpansion. The existence of such a phenomenon is consistent with the known presence of selective degrees of vasospasm in other vascular beds in preeclampsia (for example, normal pulmonary vascular resistance); however, selective renal arteriospasm in the presence of normal systemic vascular resistance has not been previously described. The administration of hydralazine and, in patients with normal pulmonary capillary wedge pressure, cautious fluid administration resulted in resolution of the oliguric state. In Patient 8, the cardiac index was markedly elevated and systemic vascular resistance was normal; systemic hypertension appeared to be on the basis of hyperdynamic cardiac function and high cardiac output alone. It might be argued that this patient represents a different hemodynamic state than that traditionally observed in preeclampsia. However, in light of the hemodynamic response induced by volume infusion in patients in category I, it appears more probable that the hemodynamic profile of Patient 8 simply represents the result of excessive volume expansion. Indeed, following a brisk spontaneous postpartum diuresis, the hemodynamic profile of this patient returned to a more typical high systemic vascular resistance/moderate cardiac output state. In a hypertensive and oliguric patient with normal systemic vascular resistance but persistent renal arteriospasm, further afterload reduction alone, while perhaps effective in reversing oliguria, would likely fail to reduce the dangerously elevated blood pressure. Such was the case in Patient 8. However, blood pressure, cardiac output, and pulmonary capillary wedge pressure fell, and oliguria resolved following both preload and afterload reduction achieved by infusion of nitroglycerine.

Patient 9 (category III) exhibited a hemodynamic picture of depressed left ventricular function (low left ventricular stroke work index), elevated pulmonary capillary wedge pressure, and marked elevation of systemic vascular resistance. Oliguria appeared to be on the basis of decreased renal perfusion secondary to intense vasospasm and diminished cardiac output. In such patients, fluid restriction with aggressive afterload reduction is indicated.

It is evident that the oliguria in patients with preeclampsia may be associated with different hemodynamic states. Patients in categories I and III represent different extremes of the hemodynamic spectrum in-

**Table I.** Hemodynamic parameters of preeclampsia with persistent oliguria

	<i>Patient No.—category I</i>					<i>Patient No.—category II</i>			<i>Patient No.—category III</i>
	<i>1</i>	<i>2</i>	<i>3</i>	<i>4</i>	<i>5</i>	<i>6</i>	<i>7</i>	<i>8</i>	<i>9</i>
Following unsuccessful fluid challenge									
Mean arterial pressure	120	110	101	118	107	117	129	130	140
Pulmonary capillary wedge pressure	7	3	4	5	1	9	10	18	18
Central venous pressure	6	1	0	2	2	5	9	9	3
Cardiac index	3.5	4.2	3.5	4.1	4.0	4.8	5.1	6.4	2.6
Systemic vascular resistance	1378	1245	1393	1497	1135	1093	1043	921	2790
Left ventricular stroke work index	54	90	54	66	59	88	94	89	33
Therapy	Fluid	Fluid	Fluid	Fluid	Fluid	Fluid/hydralazine	Fluid/hydralazine	Nitroglycerine	Hydralazine
Following therapy and resolution of oliguria									
Mean arterial pressure	117	123	120	107	107	110	107	105	104
Pulmonary capillary wedge pressure	11	7	11	9	11	9	15	2	8
Central venous pressure	11	5	4	—	—	8	10	—	—
Cardiac index	4.6	4.5	4.1	5.0	4.4	5.4	5.1	5.6	4.0
Systemic vascular resistance	961	1237	1367	1138	1049	884	843	910	1867
Left ventricular stroke work index	66	111	65	59	50	65	60	65	51

In eight patients, oliguria was managed during the intrapartum period. One patient became oliguric in the first few hours post partum, following a vaginal delivery with an estimated blood loss of 300 ml. The urine specific gravity exceeded 1.025 in all patients while they were oliguric. Initial hemodynamic parameters, type of intervention, and postintervention hemodynamic parameters, are listed in Table I. In all cases, therapy resulted in resolution of the oliguria, with outputs exceeding 35 ml/hr. No pulmonary edema was observed in the study group.

Oliguria in patients in category I responded to volume expansion alone. All patients in this group exhibited a significant increase in pulmonary capillary wedge pressure and cardiac index with volume infusion. In addition, a fall in systemic vascular resistance was observed in all five patients, but this change did not reach statistical significance ( $p = 0.09$ ) (Fig. 1). No significant or consistent change in blood pressure was observed, with mean arterial pressure rising in three patients and falling in two.

All infants had 5-minute Apgar scores of  $\geq 7$ . Two infants were delivered vaginally; and seven deliveries were by primary cesarean section, two for fetal distress and five for failed induction of labor. In each case the maternal postpartum course was uneventful.

### Comment

The central hemodynamic picture of most patients with severe preeclampsia involves hyperdynamic left ventricular function, variably increased systemic vascular resistance, and normal pulmonary vascular resistance compared with nonpregnant control subjects.<sup>4,7</sup> However, left ventricular function correlates inversely with systemic vascular resistance. Thus such patients may present with a spectrum of hemodynamic alterations, ranging from a high cardiac output state associated with moderate increases in systemic vascular resistance to a state of low cardiac output and left ventricular failure associated with marked elevation of systemic vascular resistance. Intravascular volume depletion is also a prominent feature of this disease process, most patients presenting with low or low-normal pulmonary capillary wedge pressure.<sup>8</sup>

Our findings suggest that in such a setting oliguria may occur on the basis of at least three different mechanisms. In patients in category I (Nos. 1 to 5), the hemodynamic profile was one of hyperdynamic left ventricular function, low to low-normal pulmonary capillary wedge pressure, and only moderate increases in systemic vascular resistance (Table I). Oliguria in these patients appeared to be on the basis of a relative intravascular volume depletion in the face of systemic ar-

# Severe preeclampsia with persistent oliguria: Management of hemodynamic subsets

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Nine patients with severe preeclampsia or eclampsia complicated by persistent oliguria failed to respond to fluid challenge and underwent pulmonary artery catheterization to guide further fluid and hemodynamic management. Three hemodynamic subsets of patients were defined. Patients in category I had low pulmonary capillary wedge pressure, hyperdynamic ventricular function, and moderate elevation of systemic vascular resistance. These patients responded to volume infusion with a decline in systemic vascular resistance, a rise in wedge pressure and cardiac output, resolution of oliguria, and no change in blood pressure. Patients in category II had normal or elevated pulmonary capillary wedge pressure and cardiac output and normal systemic vascular resistance; they responded to pharmacologic preload and/or afterload reduction. A single patient (category III) exhibited markedly elevated wedge pressure and systemic vascular resistance and depressed ventricular function. Oliguria in this patient responded to volume restriction and aggressive afterload reduction. Hemodynamic observations in patients in category II imply the presence of selective vasodilator responsive renal arteriospasm in some preeclamptic patients with oliguria. (AM J OBSTET GYNECOL 1986;154:490-4.)

**Key words:** Preeclampsia, hypertension, oliguria, pulmonary artery catheter

Oliguria is a well-known complication of preeclampsia; this diagnosis warrants classification of the disease as "severe" and mandates expeditious delivery.<sup>1</sup> Persistent severe oliguria may place the patient at increased risk of acute tubular or cortical necrosis as well as subject the patient receiving magnesium sulfate to the risk of magnesium toxicity. Fortunately, such oliguria will usually respond to a cautious fluid challenge, accompanied by delivery of the fetus. If oliguria persists despite institution of these measures, further fluid management with central hemodynamic monitoring has been advocated.<sup>2</sup> Published experience with such patients, however, is lacking. We report here our experience with nine oliguric preeclamptic patients whose urine output did not respond to a clinically adequate fluid challenge. Each patient underwent pulmonary artery catheterization to guide further fluid and hemodynamic manipulation, resulting in each case in resolution of the oliguria.

## Material and methods

The study group consists of nine patients who were cared for between September, 1983, and March, 1985,

at the Los Angeles County/University of Southern California Women's Hospital. All patients were diagnosed as preeclamptic by standard criteria of hypertension, proteinuria, and edema.<sup>1</sup> "Severe" preeclampsia was diagnosed by a blood pressure exceeding 160/110 mm Hg in five patients, by the presence of persistent oliguria in one patient, and by elevated and rising serum transaminase levels in one patient. The remaining two patients were eclamptic. In all cases, second-trimester or early third-trimester blood pressures had been documented in the normal range. Maintenance fluids consisted of crystalloid, administered at 100 to 125 ml/hr. Oliguria was defined as urine output (via an indwelling catheter) of less than 30 ml per hour for 3 consecutive hours. Patients exhibiting such oliguria received a fluid challenge consisting of 300 to 500 ml of lactated Ringer's solution or half-normal saline solution administered over 20 minutes. Patients whose urine output remained unchanged or further declined following this challenge underwent pulmonary artery catheterization to guide further hemodynamic and volume therapy. Technique of catheter placement and transducer calibration has been described previously.<sup>3</sup> Statistical analysis was via a paired *t* test.

## Results

Nine patients meeting the above clinical criteria underwent pulmonary artery catheterization. The mean age was 27 years (range, 19 to 41 years). Six patients were nulliparous and three were parous. The mean gestational age was 33 weeks (range, 30 to 37 weeks).

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sidered unlikely. The mechanism proposed as being responsible for simple pneumothorax, namely rupture of emphysematous blebs, is not likely to be the cause of Hamman's syndrome.<sup>12-14</sup> Other events reported in association with this syndrome outside of pregnancy include trauma, pulmonary infections, pulmonary neoplasms, neonatal atelectasis, asthma, Valsalva's maneuver, laryngospasm, and positive pressure respiratory assist.<sup>13</sup>

**Signs and symptoms.** Patients report a variety of symptoms including a change of voice, aphonia, dyspnea, cough, swelling often leading to gross disfigurement, sore throat, dysphagia, anxiety, hemoptysis, palpitations, chest pain, crackling sounds in the chest, and shortness of breath. Swelling occurs first in the jaws, neck, or anterior chest. Subcutaneous air has been reported to extend down to the thighs and wrists, and up to the scalp.<sup>1-16</sup> Recovery generally takes place in 3 to 14 days.<sup>15</sup>

Electrocardiographic changes include nonspecific ST and T wave abnormalities, and shifts of the electrical axis in 25% of patients with this syndrome. T wave inversion has also been reported in various leads. Wandering atrial pacemaker has also been reported.<sup>13,15,16</sup> Roentgenographic findings include pneumomediastinum, subcutaneous air, and pneumothorax.<sup>1-16</sup> Although pneumothorax is reported to be quite rare, it is possible that this feature is not adequately assessed in patients who otherwise possess the characteristics of this syndrome.<sup>2,12</sup>

The differential diagnosis includes cardiac tamponade, angina pectoris, pericarditis, dissecting aortic aneurism, mediastinitis, and pulmonary embolism.<sup>4,5</sup>

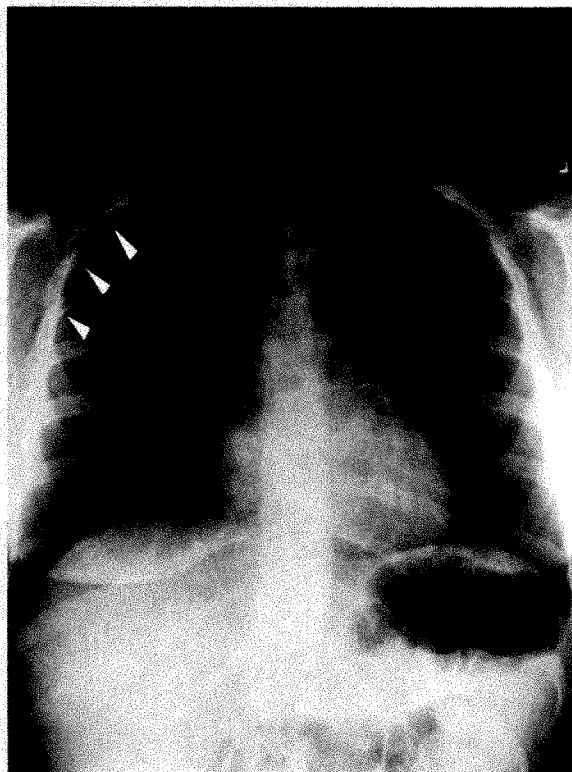
**Treatment.** Treatment of Hamman's syndrome during labor and delivery consists of sedation, oxygen, analgesics, and prevention of severe expulsive efforts during the second stage of labor by use of outlet forceps. Positive-pressure anesthesia should be avoided.<sup>2,13</sup> Several authors recommend low-forceps delivery in all subsequent deliveries in patients with a history of Hamman's syndrome.<sup>3,15</sup> I take issue with the use of prophylactic forceps in all subsequent deliveries, since a second episode of Hamman's syndrome is very unusual. Reoccurrence of this syndrome has been reported in two patients, both before 1900.<sup>3</sup> Since then, reoccurrence has not been reported.<sup>1,5,15</sup> Should the

syndrome recur in any patient, then outlet forceps can be used to shorten the second stage. Several authors have used a small incision over the suprasternal notch to decompress the subcutaneous tissues and mediastinum with excellent results in patients with severe cyanosis or dyspnea.<sup>2,5,6,13</sup> Otherwise, supportive care brings about a spontaneous resolution in the majority of cases.

In summary, pneumothorax should probably be looked for in patients who otherwise exhibit characteristics of Hamman's syndrome. Conservative care in modern times carries little risk to mother or fetus.

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**Fig. 1.** Chest roentgenogram showing right pneumothorax (arrows), pneumomediastinum (air around the great vessels), and subcutaneous emphysema.

on the right, subcutaneous air, and pneumomediastinum (Fig. 1). The pneumothorax resolved by postpartum day 3, at which time the subcutaneous emphysema was continuing to resolve. No other problems were encountered in the postpartum period.

#### Historical background

The first case report of subcutaneous emphysema without the other features of Hamman's syndrome during labor and delivery was by Simmons in 1783.<sup>3</sup> It is, however, quite possible that the midwife to the Queen of France, Louise Bourgeois, referred to this disorder when she wrote in her "Observations" in 1617, "I saw that she tried to stop crying out and I implored her not to stop for fear that her throat would swell."<sup>8</sup> Since the time of Simmons' report new cases have been periodically reported. In 1874 Haultcoer collected 13 cases and wrote a thesis on the subject. Champneys in 1875 sought to discover the etiology of this syndrome. Various collections followed until Kosmak reviewed 77 cases in 1905. Gordon in 1927 reviewed these and 53 more for a total of 130 cases. Several cases have been published since.<sup>1</sup> Most authors until the 1980s claimed that pneumothorax had never been found with the other features of Hamman's syndrome during labor and delivery.<sup>1,2,6</sup> There are several reports of isolated

spontaneous pneumothorax without the other features of Hamman's syndrome before, during, and after labor and delivery.<sup>7</sup> The first case report of Hamman's syndrome including pneumothorax during labor and delivery was in 1949.<sup>8</sup> Since then four cases have been reported, albeit two cases in the Russian literature were not confirmed by chest roentgenogram.<sup>8-11</sup> There is one case of Hamman's syndrome reported in association with hyperemesis gravidarum at 20 weeks' gestation.<sup>1</sup>

#### Case review

Hamman's syndrome with and without pneumothorax is believed to take place mostly in healthy primiparous patients with protracted labor patterns and larger than usual babies.<sup>1,3,4,8</sup> Of the 187 cases reviewed by the author the parity was stated in 169. Of these, 161 were primiparous (95%) and 8 were multiparous (5%). An analysis of cases of first-stage labor for which data were available revealed a mean of 18.3 hours (SD = 10.17 hours) from onset of active contractions to full dilation of the cervix. Mean duration of second-stage labor was 4.1 hours (SD = 2.56 hours). Fetal weight analysis yielded a mean of 3582 gm (7 pounds, 14 ounces) with a standard deviation of 410.6 gm. These data indicate that most women with this syndrome during labor and delivery are indeed primiparous and that the mean length of labor and fetal size are within normal limits.

There have been four reported maternal deaths, all before 1908. In two of the four, cyanosis and dyspnea were prominent features. Details of the other two maternal deaths are lacking.<sup>3,4</sup>

Thirteen stillborn infants and one death in the immediate postpartum period have been reported. This represents a fetal mortality of 7.4% in the 187 cases reviewed. The last fetal death associated with this syndrome took place in 1949 to an infant delivered to a woman in status asthmaticus.<sup>6</sup> The other fetal deaths occurred before 1925.<sup>3,4</sup> Four deaths occurred in infants who underwent either unsuccessful forceps attempts or craniotomy performed to effect a delivery.

**Pathophysiology.** The sequence of events leading to air in the mediastinum, subcutaneous tissues, and pleural space was investigated by Macklin and Macklin in 1944.<sup>12</sup> Alveoli whose bases are adjacent to blood vessels rupture, secondary to increased intraalveolar pressure, in conjunction with decreased vascular caliber setting up a pressure gradient into the vascular sheath. Air then dissects along the vascular sheath into the mediastinum. Air may then dissect from the mediastinum through fascial planes into more subcutaneous and retroperitoneal tissues. Air may also pass between visceral and parietal pleura, forming a pneumothorax. Other proposed mechanisms include air leaks through the buccal mucosa, trachea, and bronchi. These are con-

# Subcutaneous emphysema, pneumomediastinum, and pneumothorax in labor and delivery

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Hamman's syndrome occurs rarely in the setting of labor and delivery. In this report 187 cases of Hamman's syndrome, with and without objective evidence of pneumothorax, are summarized and the literature reviewed. Most of the women were primiparous, and first and second stages of labor were of normal duration (18.3 and 4.1 hour, respectively). Average fetal size (7 pounds, 14 ounces) was also found to be within normal limits. Signs, symptoms, and pathophysiology are reviewed and treatment discussed. (AM J OBSTET GYNECOL 1986;154:487-9.)

**Key words:** Hamman's syndrome, subcutaneous emphysema, pneumothorax, labor and delivery

The syndrome of subcutaneous emphysema and pneumomediastinum (Hamman's syndrome) is a rare complication of labor and delivery.<sup>1-6</sup> The incidence of this syndrome in labor and delivery has been estimated to be 1:2000 to 1:100,000.<sup>1</sup> Hamman originally described the syndrome in 1945 as consisting of the following: pain, subcutaneous and retroperitoneal emphysema, obliteration of the cardiac dullness, crunching sounds over the heart synchronous with the cardiac cycle (Hamman's sign), evidence of increased mediastinal pressure, dyspnea, cyanosis, engorged veins, circulatory failure, pneumothorax, and roentgenographic evidence of air in the mediastinum.<sup>2</sup> One hundred eighty-seven cases occurring during labor and delivery will be summarized from the world literature. Although many authors have stated that pneumothorax does not exist with this syndrome during labor and delivery,<sup>4,5</sup> five cases are reported to demonstrate pneumothorax. We are adding to the literature a new case of this syndrome including pneumothorax. An analysis of fetal weight and length of labor and discussion of treatment options follows.

### Case report

An eighteen-year-old girl, gravida 1, para 0, estimated date of confinement January 22, 1983, presented on January 16 with an uncomplicated prenatal

course and contractions every 2 to 3 minutes starting at 8:30 AM. The patient reported signs of blood but no rupture of membranes. Initial physical examination revealed a gravid Caucasian female in no acute distress. The lungs were clear, breath sounds symmetrical, and findings of cardiac examination were within normal limits. The abdomen was gravid, with fetal heart tones of 150 per minute. The cervix was found to be 2 cm dilated and 100% effaced, and the fetal vertex was in the -2 position. The patient achieved complete dilation by 9:10 AM. The patient pushed vigorously during the second stage of labor but had some difficulty in bringing the head down. An acute swelling was noted over the mandible on the right at 10:00 AM. Delivery of an 8 pound, 8 ounce term male was accomplished at 11:54 AM over a midline episiotomy. Apgar scores were 8 and 9 at 1 and 5 minutes, respectively. Delivery was complicated by a third-degree extension of the episiotomy. After delivery the patient received 5 mg of morphine sulfate and local Xylocaine for the episiotomy repair, when she suddenly developed difficulty breathing described as "nasal stuffiness." Swelling became apparent in the left supraorbital area, and the swelling over the right face became more pronounced. A rash was noted over the thorax. Some relief was provided by 50 mg of diphenhydramine given intramuscularly. On postpartum day 1 the patient complained of occasional shortness of breath and a "crackling" sensation when swallowing. Examination showed an ecchymotic right infraorbital area, subcutaneous emphysema in the face, neck, upper chest, and upper back, and a crunching sound synchronous with the cardiac cycle. Breath sounds were decreased on the right. A chest roentgenogram revealed a 30% pneumothorax

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of failure of the obstetricians to intervene, the latter might have run the risk of criminal liability for failure to provide medical care for the unborn child.<sup>15</sup>

While the courts have tilted in favor of the fetus late in gestation, the rights of the fetal patient earlier in gestation are not quite as clear. *Roe v Wade*, 410 US 113, 93 S Ct 705 (1973) established the state's interest in protecting the life of the unborn fetus once it has reached viable age. There is little case law, however, on the rights of the fetus earlier in gestation. In *Taft v Taft*, 388 Mass 331 (1983) a woman who had been delivered of one child at 7 months and whose three other children were delivered at term following cerclage refused a fourth operation because of religious convictions. The state supreme court reversed a lower court's order for the woman to have the operation, arguing that no evidence of the degree of likelihood that the pregnancy would carry to term without the cerclage had been presented. Thus the fundamental issue of the previable fetus' rights has not yet been addressed. Another legal commentary holds that if case law and statutes establish the fetus as an independent patient, the right of a mother to refuse fetal therapy may be foreclosed. Based on historic legal recognition of fetal rights (for example, property) and the feeling that technology has transformed the fetus into a patient, the author concludes that the fetus has a right to necessary medical care.<sup>16</sup>

It had been held that a parent of dependent children whose life is endangered by the refusal of blood transfusion because of religious belief may be forced to accept the unwanted treatment. In *Application of the President and Directors of Georgetown College Hospital*, 331 F2d 1000 (1964) a court-ordered transfusion was administered to a 25-year-old woman who had lost two thirds of blood volume. The court reasoned that the patient had a responsibility to the community to care for her 7-month-old child. Further, the state would not allow a parent to abandon a child, and her death due to refusal of blood would constitute the ultimate of voluntary abandonments and child neglect. However, a more recent decision (*In Re Osborne*, 294 A2d 372 DC [1972]) held that since the patient had, through material provision and family and spiritual bonds, provided for the future well-being of his two children, there was no compelling state interest that justified overriding the patient's decision not to have a transfusion.

The courts have not been consistent in ordering transfusions for patients with no dependents. In supporting a lower court decision to transfuse an unconsenting 22-year-old woman, the New Jersey Supreme Court reasoned that unless the risk of transfusion carried with it the risk of death or severe infirmity, the state's obligation to protect the lives of its citizens su-

perceded the patient's right to refuse transfusion (*John F. Kennedy Memorial Hospital v. Heston*, 58 NJ 576, 279 A2d 670 [1971]). However, in a similar case, the Supreme Court of Illinois expunged a lower court order for conservatorship and transfusion of an adult Witness. It was the court's opinion that the patient's right to exercise her religious beliefs should be limited only when such action endangers the public health, welfare, and morals (*In Re Brooks Estate*, 32 Ill 2d 361, 205 NE2d 435 [1965]).<sup>17</sup>

In all of the cases cited above sufficient time was available to obtain judicial resolution of the transfusion issue. The physician who, under urgent or emergency circumstances, opts to transfuse a patient despite her refusal does so at risk of legal scrutiny. Factors such as the level of consciousness and competence of the patient, the proximity of time of refusal to time of the emergency, and the availability of appropriate persons (for example, family members) to participate in decision-making would undoubtedly be weighed by the court in deciding the rectitude of the physician's actions. Not every case of life-threatening hemorrhage may be anticipated. The practitioner should become familiar with the manner in which the local court will conduct a telephone hearing in dealing with emergencies. In the instance of intrauterine pregnancy when the patient presents for routine prenatal care, early legal counsel, as in the Raleigh Fitkin—Paul Morgan Hospital case, may clarify the potential civil and criminal liability issues for the patient and her attending physician and hospital.

There are other areas in obstetrics and gynecology for which no case law exists. These include refusal of anti-D globulin prophylaxis and intrauterine fetal transfusion. Undoubtedly as statutes and cases regarding fetal rights evolve and as technology advances, these and similar issues will be clarified.

In conclusion, while not declining medical care, Jehovah's Witnesses will usually refuse blood or blood products. Theologically acceptable alternatives include plasma volume expanders and extracorporeal hemodilution. Deciding on the point at which only blood will be lifesaving is a matter of critical clinical judgment. In following the pregnant Jehovah's Witness, the physician is advised as a precautionary measure to prospectively involve hospital legal counsel early in the course of her care.

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States, the perfluorochemicals are a class of volume expanders that do have oxygen-carrying capacity. Unlike hemoglobin, which carries oxygen in combination, the perfluorochemicals carry oxygen in solution and will increase oxygen content only under conditions of high inspired oxygen tension.<sup>7</sup> Thus a severely anemic patient might require several days of perfluorochemical infusions accompanied by high inspired oxygen tensions until the endogenous hemoglobin level is restored. Two cases of severely anemic postpartum patients successfully treated with perfluorochemicals have been reported.<sup>8</sup>

Most Witnesses will accept the extracorporeal circulation of their own blood as long as it is kept in physical continuity with their circulatory systems. Blood is a non-Newtonian fluid whose viscosity depends on the concentration of red cells in plasma. As the hematocrit falls, the viscosity decreases, resulting in decreased peripheral vascular resistance, increased venous return, and increased cardiac output. If blood is diluted to a hematocrit of 20%, the increase in cardiac output allows maintenance of 100% delivery of oxygen to the tissues.<sup>9</sup> Systems employing extracorporeal hemodilution have been used in surgical procedures for Witnesses. Collected blood is diluted with volumes of crystalloid equal to two to three times the amount of blood withdrawn and then reinfused. Since hemodiluted blood is lost during an operation, there is a reduction in the absolute number of red cells lost during the operative procedure.<sup>10,11</sup> Extracorporeal circulation with use of equipment primed with lactated Ringer's solution has been used for major cardiovascular surgery in Jehovah's Witnesses. The authors of a study of over 500 such operations report that, of their 51 deaths, either blood loss or perioperative anemia were contributing factors in 15 cases. The authors conclude that such operations may be undertaken with an acceptably low risk.<sup>12</sup>

A retrospective review of major obstetric and gynecologic operations demonstrated no significant differences in perioperative morbidity between the Jehovah's Witnesses and their non-Witness controls. While there were no deaths, one of the controls with an ectopic pregnancy required 4 U of blood to raise the hemoglobin level to 7.5 gm/dl.<sup>13</sup> It seems likely that this patient might have died had blood transfusion been withheld. Indeed, the hemorrhagic death of a Witness with a ruptured ectopic pregnancy despite restoration of normovolemia has been reported.<sup>14</sup>

It thus becomes evident that despite plasma volume expansion, only the administration of blood may be lifesaving in some instances of acute massive hemorrhage. Such bleeding may be encountered in ectopic pregnancy, placenta previa, abruptio placentae, and

postpartum hemorrhage. Determining that point at which only blood will be lifesaving is a matter of critical clinical judgment. There are potential legal consequences of either administering blood to or withholding blood from an adult Witness who refuses transfusion. Variables may include the patient's pregnancy status, the presence of minor dependent children, the proximity of time of refusal to time of operation, and the patient's competence when refusing.

A physician who knowingly administers blood to an unconsenting, competent adult may be liable for civil damages. If the forced transfusion is perceived by the court as having saved the patient's life, alleged damages for personal invasion may be only nominal. However, factors such as depth of religious belief and the shunning by family or church members of the person who has been transfused may increase the amount of punitive damages. Punitive damages may seem unlikely when the physician was following his or her oath to preserve life. To date there have been no recorded suits for damages by Jehovah's Witnesses who have been transfused forcefully. While one reason for this may be pretrial settlements, an alternative explanation may be that Witnesses believe their injuries cannot be compensated for by money. Physicians and hospitals should not ignore, however, the possibility of substantial legal action against them under federal civil right statutes when the beliefs of a religious minority are ignored.

In the evolving area of fetal versus maternal rights, a body of case law has emerged making the mother's right of refusal subordinate to the viable fetus' right to live. In *Ruleigh Fitkin—Paul Morgan Hospital v Anderson*, 42 NJ 421, 201 A2d 537, *cert denied*, 377 US 985 (1964), the plaintiff hospital sought authority to transfuse a Jehovah's Witness at 32 weeks' gestation should it be necessary to save her life and that of the unborn child. The court ordered the transfusion, declaring that an unborn child is entitled to the law's protection. A similar decision was reached in *Jefferson v Griffin Spaulding Memorial Hospital*, 247 Ga 86, 274 SE2d 457 (1981), in which case a woman at 39 weeks' gestation with a sonographically diagnosed total placenta previa refused cesarean section for religious reasons. In ordering the operation the court noted that the First Amendment embraced two concepts: freedom to believe and freedom to act. The first is absolute, but the second is limited, in this instance by the child's right to live. In another case, in which for apparently personal reasons a mother in labor near term refused to consent to an operation for fetal distress, a hearing was convened at bedside. Attorneys for the mother and the fetus were appointed, the case argued, and a decision rendered to perform the cesarean section. In reporting this case, the authors comment that had the fetus died because

# Blood transfusion and Jehovah's Witnesses: Medical and legal issues in obstetrics and gynecology

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Jehovah's Witnesses are members of a religious denomination whose beliefs prohibit the use of blood or blood products. Plasma volume expanders and extracorporeal hemodilution of the patient's own blood are theologically acceptable. Acute massive hemorrhage in which only blood is lifesaving may be encountered in obstetrics and gynecology. Either withholding or administering blood in such circumstances may have legal consequences for the physician and hospital. Factors to be considered include fetal viability, the presence of dependent children, and rules of informed consent. Whenever possible, the potential for transfusion should be anticipated and clearly discussed with the patient. When appropriate, the physician and hospital should move rapidly to obtain judicial resolution. (AM J OBSTET GYNECOL 1986;154:483-6.)

**Key words:** Jehovah's Witnesses, transfusion, hemorrhage

Founded in 1884 as a small religious community in Pennsylvania, Jehovah's Witnesses now number in excess of 2 million members worldwide, of which some 650,000 reside within the United States.<sup>1</sup> Prior to July 1, 1945, Witnesses were not explicitly prohibited from receiving blood transfusions. On that date an article in *The Watchtower*, the official journal of the Jehovah's Witnesses, forbade the taking of blood into the body, on penalty of loss of eternal life in God's kingdom. The biblical references cited to support this new doctrine pertain primarily to the oral ingestion of blood.<sup>2</sup> It is unclear why the proscription of blood was promulgated on that date and whether it represented the decision of the governing board of the denomination or of an individual. It is likewise unclear why the transfusion of blood is forbidden, while certain blood components and organ transplantation are not expressly prohibited. What is clear is that this proscription sets the Witnesses apart from most other religious communities, in that adherence to doctrine potentially jeopardizes the temporal life of the believer. It might also potentially pit the Witness patient against her physician for whom blood transfusion, when indicated, is an accepted medical procedure. Some physicians, though, believe that their refusal can be accommodated in accord with the

standard of practice that has built up for treating patients of this group.<sup>3</sup>

The physician caring for the Jehovah's Witness is thus faced with a series of ethical, medical, and legal dilemmas. Obstetricians may face an additional dilemma in the form of conflicting responsibilities to their maternal and fetal patients. Furthermore, the lack of predictability of sudden massive hemorrhage in obstetrics and gynecology may force the physician to choose rapidly from among a variety of management options, each of which has its potential medical and legal consequences. It is the purpose of this paper to review those options and to discuss their implications for clinical practice.

As a rule, Jehovah's Witnesses will refuse transfusion of whole blood, packed red cells, plasma, and platelets. This prohibition does not extend to such components as albumin, immune serum globulin, and antihemophilic preparations. The use of the latter is left to the conscience of the individual member.<sup>4</sup> Despite the doctrinal stance of the sect regarding blood and blood products, there may be variation among individual members in the degree of observance of that doctrine. In response to a confidential questionnaire, some members of a Witness congregation indicated a willingness to accept plasma, while one was willing to accept even autotransfusion.<sup>5</sup>

Witnesses do not object to such plasma volume expanders as crystalloids, dextrans, and hydroxyethyl starch.<sup>6</sup> While none of these has significant oxygen-carrying capacity, under conditions of acute blood loss they may prevent tissue hypoxia and acidosis by maintaining peripheral perfusion.

Although not available for clinical use in the United

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earlier recognition of some human teratogens—the real purpose of case reports.

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example is the case report indicating that 5-fluorouracil caused multiple congenital anomalies.<sup>16</sup> Although multiple animal studies have shown 5-fluorouracil to be a teratogen, there are no other reports of human fetal exposure, and additional experience will be needed to confirm (or disprove) this agent's teratogenicity.

### Evaluation of case reports

In evaluating the significance of an individual case report it must be remembered that case reports only suggest an association, they never prove cause and effect. To establish an agent as a human teratogen, other supporting data are necessary. The great value of case reports is in the suggestion of a syndrome or malformation to be looked for prospectively or in case-cohort studies. They also provide the impetus to search for similar case reports, to retrospectively examine birth defect registry data, and to widen the investigation to include drugs with similar characteristics.

Animal data are a useful source of corroborating evidence, although species differences must be taken into account. For example, caffeine, acetylsulfacylic acid, and sulfonamides are each potent teratogens in animals but not in humans under conditions of normal human use. More attention must be paid to comparable dosages of a specific teratogen during animal studies, and plasma levels of the suspected agent must be monitored. This is an area that deserves more attention from the teratology research community. The use of agents in animal studies at dosage levels hundreds of times greater than that found in human usage does not provide for reasonable interpretation of the data as they apply to humans. Also helpful is the use of multiple species to enhance reliability and the use of a sufficient number of test animals to achieve statistical significance.

The examination of the pattern of malformations described is helpful in the evaluation of case reports. A proper diagnosis is crucial to rule out a nondrug effect or a known genetic defect. The amniotic band defect thought initially to be due to erythromycin is an example of this. The uniqueness of a malformation may aid in the recognition of a teratogenic effect. The severity and rarity of the phocomelia associated with thalidomide and the rarity of the nasal hypoplasia associated with warfarin were instrumental in the early identification of teratogenic effect. Other helpful information to be gained by observing the pattern of malformations includes whether the agent affects the same region or tissue that is known to be affected in adults and whether the period of exposure occurred at the time of organogenesis of the affected system.

### Comment

The difficulty in evaluating case reports in teratology should not belie their importance. They often serve as the first indicator of a causal relationship. Assignment of cause and effect, however, is difficult for several reasons: (1) the large number of suspected agents, 1353 in the *Catalog of Teratogenic Agents*<sup>1</sup>; (2) the 900 different drugs reported ingested by pregnant women in the National Institutes of Health Collaborative Perinatal Study<sup>2</sup>; (3) the ingestion of multiple agents by most pregnant women compared to the small percentage of birth defects caused by teratogens. Even with these difficulties there is a need for careful evaluation of case reports, both to avoid alarm in the lay and scientific communities and to further decrease perinatal morbidity and mortality.

The individual case report can often lead to the collection of a small prospective series of exposed pregnancies. How long it will take to accumulate such a series depends on how commonly the substance in question is either used therapeutically or abused. For example, in the case of isotretinoin, the teratogenicity of large doses of vitamin A in animals and humans was well documented before isotretinoin was licensed. Even though the drug was clearly contraindicated in pregnancy, its use was so common that many cases of inadvertent exposure occurred, allowing recognition of the frequency and severity of the malformation syndrome. Thus the case report can serve to alert the medical community to a possible association and remind physicians that the benefit of any agent administered to the pregnant woman or woman in her childbearing years must be carefully weighed against its teratogenic potential.

Many existing reference sources exist to aid the practicing physician. In addition to a number of texts and journal articles discussing drug usage in pregnancy, the Environmental Teratology Information Center provides a large data base whose records are computer searchable. The authors would like to propose another mechanism for attracting and organizing case reports of possible human teratogenic associations. This would take the form of a "letters section" in a journal in this field of interest. Contributors would be urged to submit detailed letters to allow critical editorial review of case reports describing human teratogenicity. This would provide (1) a centralized repository, which would correct much of the current problem of report distribution; (2) editorial review to be certain that all the necessary data were provided; (3) easier access to the printed page than is provided by refereed journal articles. This would encourage the reporting of observations of practicing physicians and might well provide

complicates the collection and evaluation of evidence regarding a possible teratogen and the significance of a single case report.

There are also many compounding factors that make it difficult to assign specific defects or constellations of defects to specific drugs: (1) the drug may be administered as therapy for an illness that itself causes malformations; (2) the fetal malformation may cause maternal symptoms, which are treated with a specific drug; (3) the drug may inhibit the abortion of an already malformed infant; (4) the drug may commonly be employed in combination with a second drug, which causes a malformation. The biology of the situation also inhibits clear assignment of cause and effect. Many genes and different agents may produce identical defects because there is a limited variability in what nonlethal changes can occur in developing cells, tissues, and organs. These events or mechanisms lead to a limited number of pathways resulting in birth defects and therefore to a certain commonality to the malformation produced. Thus even when a defect occurs after exposure to a suggested teratogen, due consideration must be given to the possibility that the defect is the result of a particular gene or constellation of genes or to exposure to something other than the suspected teratogen.

In spite of the above difficulties in drawing conclusions, many agents have been well proven to be teratogens. Many other agents initially thought to be teratogens have been proved not to be teratogens, while other suspected drug associations are still awaiting further investigation.

#### Case report—suggested associations

**Associations established.** The best evidence for the value of case reports in establishing human teratogenesis comes from considering what is probably the most infamous human teratogen. Lenz<sup>6</sup> and McBride<sup>7</sup> simultaneously reported several case reports of limb-reduction malformations in newborn infants resulting from maternal ingestion of a sedative, thalidomide, early in pregnancy. Multiple confirmatory reports followed these initial reports. The highly recognizable syndrome of rare malformations caused by this agent was instrumental in aiding its rapid recognition. If the drug had caused cleft palate, a much more common birth defect, the relationship would have been much more difficult to establish. The thalidomide tragedy has served to illuminate the significance of teratology and has had obstetric, legal, pharmaceutical, and governmental regulatory repercussions.

Another well-established teratogen whose association with anomalies was first due to a case report was war-

farin. A case report observed that a pregnant woman with a prosthetic mitral valve who was taking warfarin gave birth to a child with hypoplastic nasal structures.<sup>8</sup> The authors suggested the association between warfarin use in the mother and the birth defect in the offspring. Two earlier reports of women with prosthetic cardiac valves who were treated with warfarin during pregnancy producing malformed infants had failed to draw conclusions or to alert the medical community.<sup>9,10</sup> In the early 1970s many additional abnormal infants exposed to warfarin in utero were studied, allowing more complete characterization of the warfarin embryopathy.<sup>11</sup>

Another drug that has been recently shown to be a human teratogen is isotretinoin, licensed in September 1982 for the treatment of severe cystic acne. An initial case report by Rosa<sup>12</sup> in 1983 reported eight cases of hydrocephalus associated with microtia and/or microphthalmos in isotretinoin-exposed fetuses. This embryopathy was further characterized in a large multicenter study, which revealed a malformation rate of 18% in newborn infants, similar to the 20% malformation rate with thalidomide exposure.<sup>13</sup>

**Associations disproved.** Bendectin, an antiemetic preparation and until recently one of the most common drugs ingested during pregnancy, has come under much scrutiny as a possible teratogen. A case report, manifesting itself as a legal suit, alleged that Bendectin caused a limb reduction defect.<sup>14</sup> A number of retrospective and prospective studies have failed to show that Bendectin is a human teratogen. In spite of this, the drug has been taken off the market by the manufacturer because of pending legal cases. Litigation can thus serve to implicate an agent, stressing the need for adequate scientific evaluation. Although it is difficult, and perhaps even impossible, to prove an agent is not teratogenic, the association of Bendectin and limb reduction defects should be interpreted as fortuitous.

Another drug that was thought to be a teratogen was erythromycin.<sup>15</sup> Examination of the published photograph, however, indicated that the affected fetus had the amniotic band syndrome, a recognized sporadic malformation complex. Therefore the anomalies should not be attributed to the erythromycin exposure. This example demonstrates the need for careful evaluation before publication of a case report. Pictures, x-ray films, an autopsy report, and if possible, a karyotype of the affected fetus or neonate should be examined by a dysmorphologist to eliminate other causes that may have produced the birth defect(s).

**Associations under consideration.** The majority of teratogenic associations suggested by case reports in the medical literature remain to be confirmed. One such

## CLINICAL SECTION

### Clinical Opinion

# The value of case reports in human teratology

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Birth defects caused by human teratogens are an important and potentially preventable cause of perinatal morbidity and mortality. Case reports provide an initial suggestion that a specific agent may be a human teratogen and provide the basis for further study. This review discusses the importance of case reports in human teratology and provides guidance in evaluating new case reports. (AM J OBSTET GYNECOL 1986;154:479-82.)

**Key words:** Drugs, case reports, teratology, birth defects

Concurrent with the advances in obstetric and neonatal care that have decreased perinatal morbidity and mortality rates, there has been increased lay and scientific interest in birth defects. In 1900 the perinatal mortality rate in the United States was 150 per thousand live births with five (3.3%) of these losses resulting from birth defects. By 1978, when the perinatal mortality rate had fallen tenfold to 15 per thousand live births, the same five deaths from birth defects accounted for one third of the losses. This has led to a focusing of attention on the problem of birth defects and on teratology as one aspect of that problem. Additionally, as western civilization has experienced a "back to nature" resurgence, people have become more suspicious about the teratogenic potential of environmental and therapeutic agents.

Depending on the population surveyed and the definitions used, birth defects occur at a 3% to 9% incidence rate. Although, as pointed out by Wilson,<sup>1</sup> only 2% to 3% of developmental defects are known to be caused by drugs or environmental agents (65% to 70% are of unknown origin), these agents are potentially avoidable once they are identified.

Identifying these agents, however, is a major problem, the scope of which is revealed by the list of 900 different drugs taken by pregnant women in the National Institutes of Health Collaborative Perinatal

Study.<sup>2</sup> In addition, in a survey performed in 1973 pregnant women took an average of almost four drugs, excluding nutritional supplements, with only 20% of pregnant women abstaining from drug usage.<sup>3</sup> It is perhaps even more significant that 40% of these women took medication during the first trimester of pregnancy, and approximately one half the total drug consumption during pregnancy occurred during the period of organogenesis.

Many initial reports of teratogenic agents have been published as case reports. These case reports generate significant alarm in both the lay and scientific communities, emphasizing the importance of their objective evaluation. The lay press is quick to report on a publication regarding a possible teratogen and slow to give equal space to the article disproving the association. This may result in the nonuse of a needed therapeutic agent by some patients frightened by the publicity. Also, the potential for litigation every time a newborn infant has a birth defect demands careful review and interpretation of these studies.

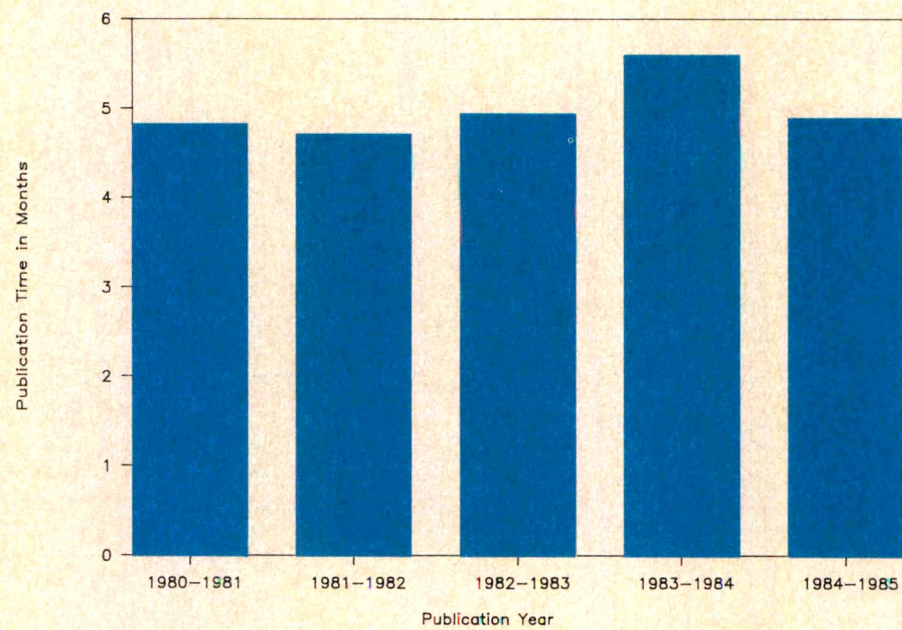
The evaluation of human teratogenicity is further complicated by the large number of agents implicated as teratogens. Shepard,<sup>4</sup> in his *Catalog of Teratogenic Agents*, lists 1353 drugs or environmental agents that have been implicated. Another difficulty in this evaluation is demonstrated in a recent review of human teratogens in which the author examined his reference sources.<sup>5</sup> These sources were in multiple languages with only 19% of the articles published in obstetric journals, 12% in teratology and genetic publications, and 9% in pediatric journals while 39% appeared in general medical journals and 12% in specialty publications other than obstetrics or pediatrics. This diversity further

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**American Journal of Obstetrics and Gynecology  
Comparison of Publication Time of Regular Articles  
(Excluding Society Articles)**

**Reporting Year****Yearly Average**

1980-1981

4.83

1981-1982

4.72

1982-1983

4.95

1983-1984

5.60

1984-1985

4.90



## EDITORIAL

### Summary of analysis of published articles

September 1, 1984, through August 31, 1985

Each year a retrospective analysis of the articles published in the JOURNAL is presented to the readers and also to the Editorial Board and the Advisory Committee on Policy. During the past sixteen years we have compiled data on the classification, category, and type of articles accepted and published. A comparison of the data for the last two years is presented below. A comparison of publication time in the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY of regular articles (excluding society articles) for 1980-1981 through 1984-1985 is shown graphically on page 478. Readers and authors should note the time from acceptance to publication was 4.90 months for the period analyzed in 1985.\*

\*Publication time of articles for regular sections from article acceptance: 1982, 4.72 months; 1983, 4.95 months; 1984, 5.60 months, and 1985, 4.90 months.

	1984			1985		
	No. of articles	Pages	Average length per article	No. of articles	Pages	Average length per article
<b>Classification of article:</b>						
Clinical	302	1148		273	1175	
Clinical Investigation	231	1185		206	1059	
Basic Research	103	544		115	619	
<b>Total</b>	<b>636</b>	<b>2877</b>		<b>594</b>	<b>2853</b>	
<b>Category of articles:</b>						
Obstetrics	226	990		230	1080	
Gynecology	220	1041		188	948	
Fetus, Placenta, Newborn	185	817		175	819	
Education	5	29		1	6	
<b>Total</b>	<b>636</b>	<b>2877</b>		<b>594</b>	<b>2853</b>	
<b>Composition of JOURNAL based on type of article:</b>						
Regular	306	1554	5.1	317	1653	5.2
Society	163	979	6.0	145	862	6.0
Brief Communication/Case Reports	150	207	1.4	103	169	1.6
Current Development	9	97	10.8	6	64	10.7
Current Investigation	2	9	4.5	8	25	3.1
Clinical Opinion	6	31	5.2	15	80	5.3
<b>Total</b>	<b>636</b>	<b>2877</b>		<b>594</b>	<b>2853</b>	

*Continued*



## IN BRIEF:

**TRIPHASIL®**—6 brown tablets containing 0.050 mg levonorgestrel with 0.030 mg ethinyl estradiol; 5 white tablets containing 0.075 mg levonorgestrel with 0.040 mg ethinyl estradiol; 10 light-yellow tablets containing 0.125 mg levonorgestrel with 0.030 mg ethinyl estradiol (7 light-green tablets containing inert ingredients are included in the 28-day regimen)—Triphasic regimen.

**Indications and Usage**—TRIPHASIL® is indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OC's) as a method of contraception.

**Contraindications**—OC's should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep-vein thrombophlebitis or thromboembolic disorders. 3. Cerebral-vascular or coronary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Known or suspected pregnancy (see Warning No. 5). 8. Benign or malignant liver tumor which developed during use of OC's or other estrogen-containing products.

## Warnings

**Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.**

The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. **Thromboembolic Disorders and Other Vascular Problems**—An increased risk of thromboembolic and thrombotic disease associated with use of OC's is well established. Three principal studies in Great Britain and 3 in the U.S. have demonstrated increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OC's are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.

**CEREBROVASCULAR DISORDERS**—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

**MYOCARDIAL INFARCTION (MI)**—An increased risk of MI associated with the use of OC's has been reported, confirming a previously suspected association. These studies, conducted in the UK, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of pre-eclamptic toxemia) the higher the risk of developing MI, regardless of whether the patient was an OC user or not. OC's, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to MI) are about twice as likely to have a fatal MI as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal MI compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, amount of smoking is also an important factor. In determining importance of these relative risks, however, baseline rates for various age groups must be given serious consideration. Importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; quite likely the same synergistic action exists, but perhaps to a lesser extent.

**RISK OF DISEASE**—In an analysis of data derived from several national adverse-reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to dose of estrogen in OC's. Preparations containing 100 mcg or more of estrogen were associated with higher risk of thromboembolism than those containing 50-80 mcg. Their analysis did suggest, however, that quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the U.S.

**ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES**—A large prospective study carried out in the UK estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OC's according to age, smoking habits, and duration of use. Overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), risk being concentrated in older women, in those with long duration of use and in cigarette smokers. It was not possible, however, to examine interrelationships of age, smoking, and duration of use, nor to compare effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess magnitude of relative risk for this younger group. Available data from a variety of sources have been analyzed to estimate risk of death associated with various methods of contraception. Estimates of risk of death for each method include combined risk of contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OC's) plus risk attributable to pregnancy or abortion in event of method failure. This latter risk varies with effectiveness of method. The study concluded that mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OC's in women over 40 who smoke. Lowest mortality is associated with condom or diaphragm backed up by early abortion. Risk of thromboembolic and thrombotic disease associated with OC's increases with age after about 30 and, for MI, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of pre-eclamptic toxemia, and especially cigarette smoking. Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A 4- to 6-fold increased risk of postoperative thromboembolic complications has been reported in OC users. If feasible, OC's should be discontinued at least 4 weeks before surgery of a type associated with increased risk of thromboembolism or prolonged immobilization.

**PERSISTENCE OF RISK OF VASCULAR DISORDERS**—Findings from one study in Britain involving cerebrovascular disease and another in the U.S. concerning MI suggest an increased risk of these conditions in users of OC's persists after discontinuation of the OC's. In the British study, risk of cerebrovascular disease remained elevated in former OC users for at least 6 years after discontinuation. In the U.S. study, increased risk of MI persisted for at least 9 years in women 40 to 49 years old who had used OC's for 5 or more years. Findings in both studies require confirmation since they are inconsistent with other published information.

2. **Ocular Lesions**—There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with use of OC's. Discontinue OC's if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. **Carcinoma**—Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in OC's, have been noted to increase incidence of mammary nodules, benign and malignant, in dogs. In humans, 3 case-control studies have reported an increased risk of endometrial carcinoma associated with prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OC's. Of cases found in women without predisposing risk factors (e.g., irregular bleeding at the time OC's were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These are no longer marketed. No evidence has been reported suggesting increased risk of endometrial cancer in users of conventional combination or progestogen-only OC's. Several studies have found no increase in breast cancer in women taking OC's or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women on OC's, found an excess risk in subgroups of OC users with documented benign breast disease. Reduced occurrence of benign breast tumors in users of OC's has been well documented. In summary, there is at present no confirmed evidence from human studies of increased risk of cancer associated with OC's. Close clinical surveillance of all women on OC's is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or with breast nodules, fibrocystic disease, or abnormal mammograms should be monitored with particular care if they elect to use OC's.

4. **Hepatic Tumors**—Benign hepatic adenomas have been found to be associated with use of OC's. One study showed that OC's with high hepatic potency were associated with higher risk than lower potency OC's. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users. Data studies relate risk with duration of use of OC's, the risk being much greater after 4 or more years' use. While hepatic adenoma is rare, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women on OC's. Relationship of these drugs to this type of malignancy is not known.

5. **Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring**—Use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have increased risk of developing in later life a form of vaginal or cervical cancer ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence now that OC's further enhance risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OC's. Furthermore, 30 to 90% of such exposed women have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with use of other estrogens, it cannot be presumed they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with use of sex hormones, including OC's, in pregnancy. One case-control study estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OC's, hormonal withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some exposures involved only a few days. Data suggest that risk of limb-reduction defects in exposed fetuses is somewhat less than 1 in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence

from well-controlled studies that progestogens are effective. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OC's. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OC's is unknown. It is recommended that, for any patient who has missed 2 consecutive periods, pregnancy should be ruled out before continuing OC's. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at time of first missed period, and further use of OC's should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and advisability of continuation of the pregnancy should be discussed. It is also recommended that women who discontinue OC's with intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this. The administration of progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. **Gallbladder Disease**—Studies report increased risk of surgically confirmed gallbladder disease in users of OC's and estrogens. In one study, increased risk appeared after 2 years' use and doubled after 4 or 5 years' use. In one of the other studies, increased risk was apparent between 6 and 12 months' use.

7. **Carbohydrate and Lipid Metabolic Effects**—Decrease in glucose tolerance has been observed in a significant percentage of patients on OC's. For this reason, prediabetic and diabetic patients should be carefully observed while on OC's. Increases in triglycerides and total phospholipids have been observed in patients on OC's. Three studies were performed with Triphasil and no significant alterations in lipid metabolism were noted except for a slight increase in triglyceride levels in 1 study. Clinical significance of these findings remains to be defined.

8. **Elevated Blood Pressure**—Increase in blood pressure has been reported in patients on OC's. In some women, hypertension may occur within a few months of beginning OC's. In the 1st year of use, prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. Prevalence in users increases, however, with longer exposure, and in the 5th year of use is 2½ to 3 times the reported prevalence in the 1st year. Age is also strongly correlated with development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure on OC's. Hypertension that develops as a result of taking OC's usually returns to normal after discontinuing the drug.

9. **Headache**—Onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of OC's and evaluation of the cause.

10. **Bleeding Irregularities**—Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing OC's. In breakthrough bleeding, as in all cases of irregular vaginal bleeding, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or change to another OC may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase risk of thromboembolic disease. Women with past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuing OC's. Women with these preexisting problems should be advised of this possibility and encouraged to use other methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. **Ectopic Pregnancy**—Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. **Breast-feeding**—OC's given in the postpartum period may interfere with lactation and decrease quantity and quality of breast milk. Furthermore, a small fraction of the hormones in OC's has been identified in the milk of mothers on OC's; effects, if any, on the breast-fed child have not been determined. If feasible, defer OC's until infant has been weaned.

**Precautions**—GENERAL—1. A complete medical and family history should be taken prior to initiation of OC's. Pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Pap smear and relevant laboratory tests. As a general rule OC's should not be prescribed for longer than 1 year without another physical examination and Pap smear.

2. Under influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size.

3. Patients with history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while on OC's should stop OC's and use an alternate method to try to determine whether the symptom is drug-related.

4. OC's may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency.

5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while on OC's. If jaundice develops, OC's should be discontinued.

6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution.

7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. Clinical significance is undetermined.

8. Serum folate levels may be depressed by OC's. Since the pregnant woman is predisposed to development of folate deficiency and incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OC's, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency.

9. The pathologist should be advised of OC therapy when relevant specimens are submitted.

10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OC's.

a. Increased subcutaneous fat retention.

b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.

c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered.

d. Decreased pregnandiol excretion.

e. Reduced response to metyrapone test.

**Information for the Patient**—See Patient Package Labeling.

**Drug Interactions**—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

**Carcinogenesis**—See Warnings section for information on carcinogenesis.

**Pregnancy**—Category X. See Contraindications, Warnings.

**Nursing Mothers**—See Warnings.

**Adverse Reactions**—An increased risk of these serious adverse reactions has been associated with use of OC's (see Warnings): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies. There is evidence of an association between the following conditions and use of OC's although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients on OC's and are believed to be drug-related. Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating), breakthrough bleeding, spotting, change in menstrual flow, dysmenorrhea, amenorrhea during and after treatment, temporary infertility after discontinuance of treatment, edema, chloasma or melasma which may persist; breast changes: tenderness, enlargement, and secretion; change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening), intolerance to contact lenses. The following adverse reactions have been reported in users of OC's, and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, hemolytic uremic syndrome.

**Acute Overdose**—Serious ill effects have not been reported following acute ingestion of large doses of OC's by young children. Overdose may cause nausea, and withdrawal bleeding may occur in females.

**Dosage and Administration**—For maximum contraceptive effectiveness, Triphasil must be taken exactly as directed and at intervals not over 24 hours. (If Triphasil is first taken later than first day of first menstrual cycle of medication or postpartum, contraceptive reliance should not be placed on it until after the first 7 consecutive days of use. Possibility of ovulation and conception prior to initiation of medication should be considered.)

Any time patient misses 1 or 2 brown, white or light-yellow tablets, she should also use another contraceptive method until she has taken a tablet daily for 7 consecutive days.

For full details on dosage and administration see prescribing information in package insert.

*Triphasil*  
Levonorgestrel  
and ethinyl  
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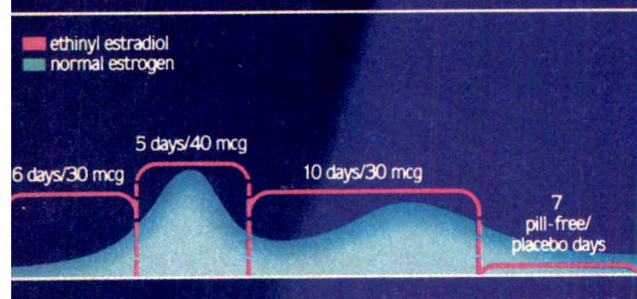
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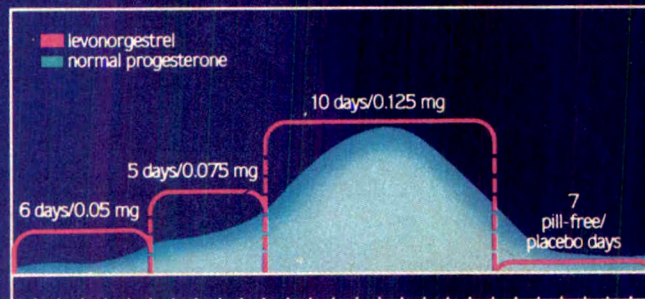
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Graham H. Letter to Editor. *Br Med J* 284:422, 1982.

See important information on following page.



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### Estimating length of manuscript

The length of text material (introduction through Comment section) in regular manuscripts accepted for publication normally ranges from 750 to 4200 words (an average of 2000 words). A 4200 word text can seldom be accepted, especially if tables and figures are included. The average manuscript of 2000 words of text with abstract, 3 tables with captions, 2 figures with legends, and references makes a 5.7 page article in the JOURNAL. The 2000 words of text alone make approximately 8 pages of manuscript typed double spaced with the required 1 inch margins (approximately 250 words per page). A table or figure that occupies both columns of half a JOURNAL page is equivalent to approximately 500 typed words in manuscript. Thus if a greater number of illustrations and tables are used, the length of the text should be adjusted accordingly.

### Requirements for special sections

**Case reports and brief clinical and basic science communications.** Limit of 700 words, 2 references. Include abstract of 50 words maximum, 3 to 5 key words/phrases for indexing purposes, and short title. If tables and/or figures are used, an equivalent number of words must be deducted from the total (see "Estimating Length of Manuscript").

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Only standard abbreviations are to be used. Consult the *Council of Biology Editors Style Manual* or the *AMA's Manual for Authors and Editors*. Abbreviations in the title are not acceptable. They should be avoided, if possible, in the abstract. In the text they should be kept to a practical minimum. The full term for which an abbreviation stands should precede its first use in the text unless it is a standard unit of measurement.

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**Acknowledgements.** Acknowledge only persons who have made substantive contributions to the study.

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Illustrations and tables should supplement, not duplicate, the text; presentation in either one or the other will suffice.

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# American Journal of Obstetrics and Gynecology

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### 2. ESTROGENS SHOULD NOT BE USED DURING PREGNANCY.

The use of female sex hormones, both estrogens and progestogens, during early pregnancy may seriously damage the offspring. It has been shown that females exposed *in utero* to diethylstilbestrol, a non-steroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated as not greater than 4 per 1000 exposures. Furthermore, a high percentage of such exposed women (from 30 to 90 percent) have been found to have vaginal adenosis, epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are precursors of malignancy. Although similar data are not available with the use of other estrogens, it cannot be presumed they would not induce similar changes. Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies, including congenital heart defects and limb reduction defects. One case control study estimated a 4.7-fold increased risk of limb reduction defects in infants exposed *in utero* to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 per 1000. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well controlled studies that progestogens are effective for these uses. If ESTRACE® (estradiol) is used during pregnancy, or if the patient becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus and the advisability of pregnancy continuation.

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**Contraindications:** Known or suspected cancer of the breast, except in appropriately selected patients being treated for metastatic disease; known or suspected estrogen-dependent neoplasia; known or suspected pregnancy (See Boxed Warning); undiagnosed abnormal genital bleeding; active thrombophlebitis or thromboembolic disorders; a past history of thrombophlebitis, thrombosis or thromboembolic disorders associated with previous estrogen use (except when used in treatment of breast or prostatic malignancy).

**Warnings:** Estrogens increase the risk of carcinoma of the endometrium (See Boxed Warning). Prescribe cautiously in women with a strong family history of breast cancer or with breast nodules, fibrocystic disease, or

abnormal mammograms. A 2- to 3-fold increase in the risk of surgically confirmed gallbladder disease has been reported in women receiving postmenopausal estrogens. There is an increased risk of thrombosis in men receiving estrogens for prostatic cancer and women for postpartum breast engorgement. If feasible, estrogen should be discontinued at least 4 weeks before surgery of the type associated with an increased risk of thromboembolism, or during periods of prolonged immobilization. Estrogens should not be used in persons with active thrombophlebitis or thromboembolic disorders or (except in treatment of malignancy) with a history of such disorders in association with estrogen use; they should be used with caution in patients with cerebral vascular or coronary artery disease and only for those in whom estrogens are clearly needed. Large doses (5 mg conjugated estrogens per day), comparable to those used to treat cancer of the prostate and breast, have been shown to increase the risk of nonfatal myocardial infarction, pulmonary embolism, and thrombophlebitis; when estrogen doses of this size are used, any of the thromboembolic and thrombotic adverse effects associated with oral contraceptives should be considered a clear risk. Hepatic adenomas should be considered in estrogen users having abdominal pain and tenderness, abdominal mass, or hypovolemic shock. Increased blood pressure occurs with use of estrogens in the menopause, and blood pressure should be monitored, especially with high doses. Diabetic patients should be carefully observed for decreased glucose tolerance. Estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases; if this occurs, the drug should be stopped and appropriate measures taken to reduce the serum calcium level.

**Precautions:** *General*—A complete medical and family history should be taken prior to initiation of therapy. Pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen, and pelvic organs, and should include a Papanicolaou smear. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination. Conditions influenced by fluid retention, such as epilepsy, migraine, and cardiac or renal dysfunction, require careful observation. Undesirable manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding, mastodynia, etc., may develop. Patients with a history of depression should be carefully observed. Pre-existing uterine leiomyomata may increase in size during estrogen use. Pathologists should be advised of estrogen therapy when relevant specimens are submitted. If jaundice develops, discontinue use while cause is investigated. Administer with caution in patients with impaired liver function, metabolic bone diseases associated with hypercalcemia, or renal insufficiency and in young patients with incomplete bone growth. The following endocrine and liver function tests may be affected by larger estrogen doses: increased sulfobromophthalein retention; increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability; increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by PBI, T4 by column, or T4 by radioimmunoassay; free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered; impaired glucose tolerance; decreased pregnandiol excretion; reduced response to metyrapone test; reduced serum folate concentration; increased serum triglyceride and phospholipid concentration.

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The 2-mg tablets contain FD&C Yellow No. 5 (tartrazine), which may cause allergic-type reactions (including bronchial asthma) in certain susceptible individuals, frequently those who also have aspirin hypersensitivity.

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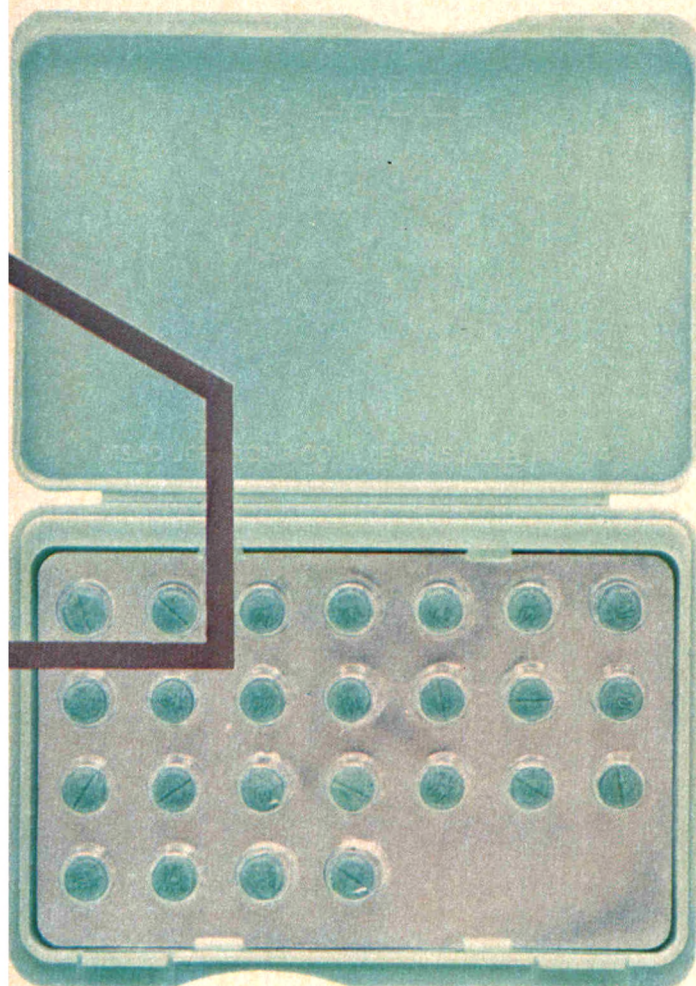
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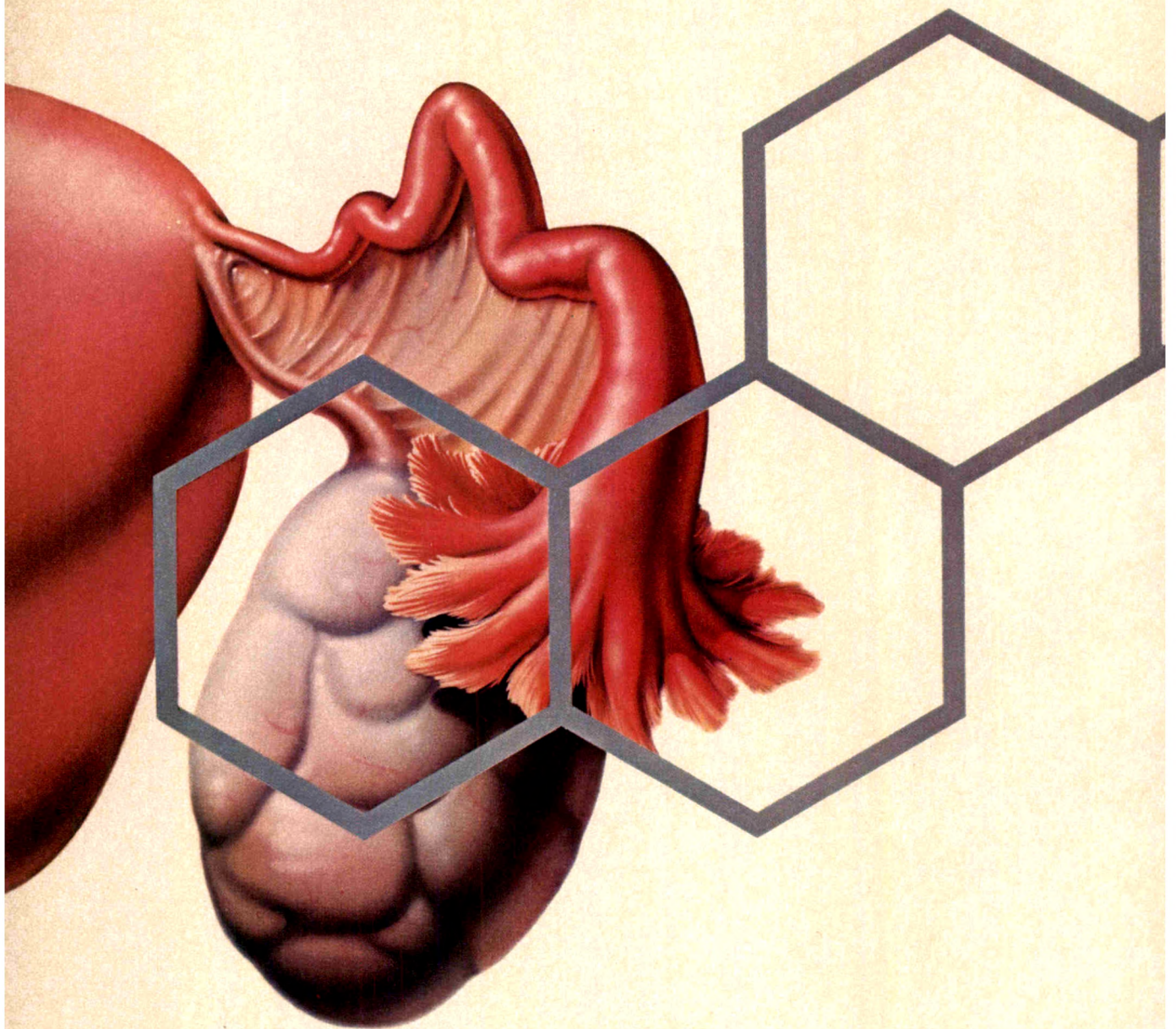
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REPLACEMENT  
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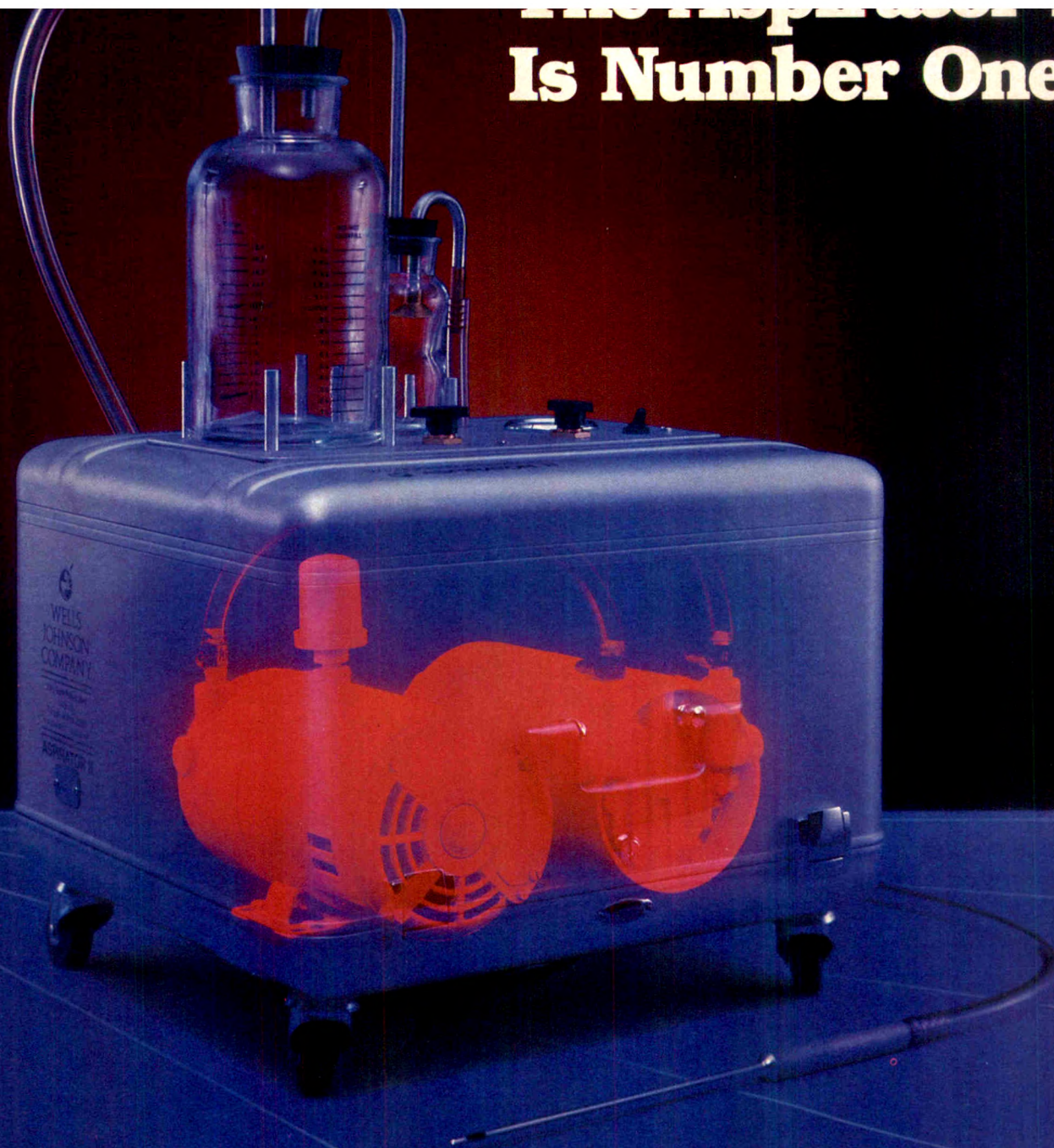
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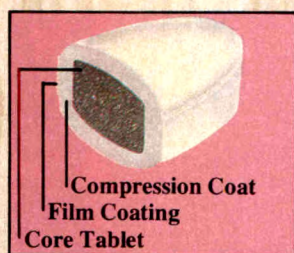
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10	400
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15	150
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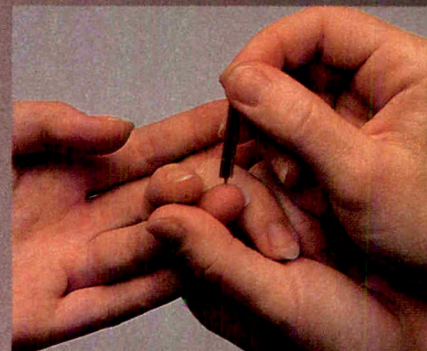
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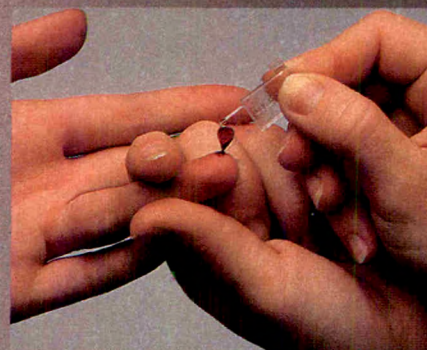
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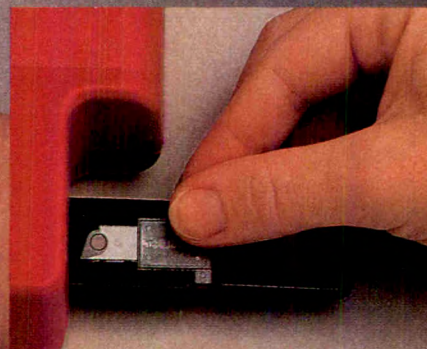
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One tablet is  
the total  
treatment



## Mycelex-G 500<sup>mg</sup> (clotrimazole) Vaginal Tablet

**INDICATIONS:** Mycelex-G 500 mg Vaginal Tablets are indicated for the local treatment of vulvovaginal candidiasis when one day therapy is felt warranted. In the case of severe vulvovaginitis due to candidiasis, longer antimycotic therapy is recommended. The diagnosis should be confirmed by KOH smears and/or cultures. Other pathogens commonly associated with vulvovaginitis, *Trichomonas* and *Gardnerella (Haemophilus) vaginalis*, should be ruled out by appropriate laboratory methods.

**CONTRAINDICATIONS:** Mycelex-G 500 mg Vaginal Tablets are contraindicated in women who have shown hypersensitivity to any components of the preparation.

**WARNINGS:** None.

**PRECAUTIONS:** If there is a lack of response to Mycelex-G 500 mg Vaginal Tablets, appropriate microbiological studies should be repeated to confirm the diagnosis and rule out other pathogens before instituting another course of antimycotic therapy.

**CARCINOGENESIS:** No long term studies in animals have been performed to evaluate the carcinogenic potential of Mycelex-G 500 mg Vaginal Tablets intravaginally. A long term study in rats (Wistar strains) where clotrimazole was administered orally provided no indication of carcinogenicity.

**USAGE IN PREGNANCY: PREGNANCY CATEGORY B:** The disposition of <sup>14</sup>C-clotrimazole has been studied in humans and animals. Clotrimazole is poorly absorbed following intravaginal administration to humans, whereas it is rather well absorbed after oral administration.

In clinical trials, use of vaginally applied clotrimazole in pregnant women in their second and third trimesters has not been associated with ill effects. There are, however, no adequate and well-controlled studies in pregnant women during the first trimester of pregnancy.

Studies in pregnant rats given repeated intravaginal doses up to 100 mg/kg/day have revealed no evidence of harm to the fetus due to clotrimazole.

Repeated high oral doses of clotrimazole in rats and mice ranging from 50 to 120 mg/kg resulted in embryotoxicity (possibly secondary to maternal toxicity), impairment of mating, decreased litter size and number of viable young and decreased pup survival to weaning. However, clotrimazole was not teratogenic in mice, rabbits and rats at oral doses up to 200, 180 and 100 mg/kg, respectively. Oral absorption in the rat amounts to approximately 90% of the administered dose.

Because animal reproduction studies are not always predictive of human response, this drug should be used only if clearly indicated during the first trimester of pregnancy.

**ADVERSE REACTIONS:** Of 297 patients in double-blind studies with the 500 mg vaginal tablet, 3 of 149 patients treated with active drug and 3 of 148 patients treated with placebo reported complaints during therapy that were possibly drug related. In the active drug group, vomiting occurred in one patient, vaginal soreness with coitus in another, and complaints of vaginal irritation, itching, burning and dyspareunia in the third patient. In the placebo group, clitoral irritation occurred in one patient and dysuria, described as remotely related to drug, in the other. A third patient in the placebo group developed bacterial vaginitis which the investigator classed as possibly related to drug.

Eighteen (1.6%) of the 1116 patients treated with Mycelex-G in other formulations in double-blind studies reported complaints during therapy that were possibly drug-related. Mild burning occurred in six patients while other complaints such as skin rash, itching, vulval irritation, lower abdominal cramps and bloating, slight cramping, slight urinary frequency, and burning or irritation in the sexual partner, occurred rarely.

**OVERDOSAGE:** No data available.

**DRUG ABUSE AND DEPENDENCE:** Drug abuse and dependence with Mycelex-G 500 mg Vaginal Tablets has not been reported.

**DOSAGE AND ADMINISTRATION:** The recommended dose is one tablet inserted intravaginally one time only, preferably at bedtime. In the event of treatment failure, that is, persistence of signs and symptoms of vaginitis after five days, other pathogens commonly responsible for vaginitis should be ruled out before instituting another course of antimycotic therapy.

**HOW SUPPLIED:** Mycelex-G 500 mg Vaginal Tablets are white, bullet shaped, uncoated tablets, coded with Miles on one side and 097 on the other, supplied as a single 500 mg tablet with plastic applicator and patient instructions.

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(RG) Applicants for this endocrinology position should be subspecialty board eligible or board certified in reproductive endocrinology, preferably with both an M.D. and Ph.D. degree, and should have extensive training and experience in laboratory aspects of molecular endocrinology and biochemistry. Individual should also be well prepared in clinical obstetrics, gynecology and infertility.

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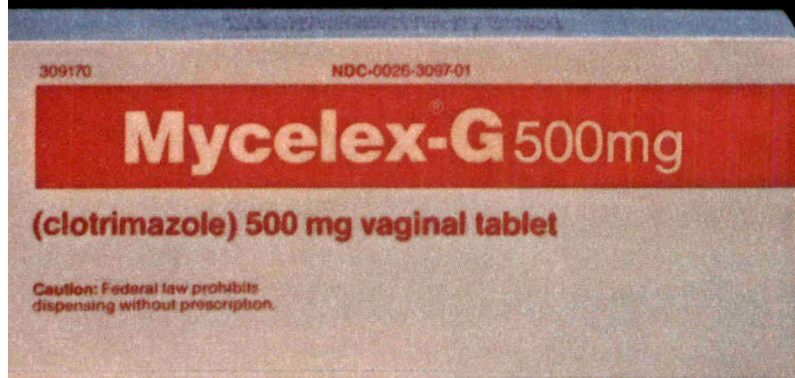
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Vaginal Tablet



In the case of severe vulvovaginitis due to candidiasis, longer antimycotic therapy is recommended.



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# American Journal of Obstetrics and Gynecology

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Bellflower and Sacramento, California

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CLEOCIN PHOSPHATE® Sterile Solution  
CLEOCIN HCl® Capsules  
(clindamycin)

#### WARNING

Clindamycin therapy has been associated with severe colitis which may end fatally. Therefore, it should be reserved for serious infections where less toxic antimicrobial agents are inappropriate, as described in the Indications Section. It should not be used in patients with nonbacterial infections, such as most upper respiratory tract infections. Studies indicate a toxin(s) produced by *Clostridia* is one primary cause of antibiotic associated colitis. Cholestyramine and colestipol resins have been shown to bind the toxin *in vitro*. See WARNINGS section. The colitis is usually characterized by severe, persistent diarrhea and severe abdominal cramps and may be associated with the passage of blood and mucus. Endoscopic examination may reveal pseudomembranous colitis.

When significant diarrhea occurs, the drug should be discontinued or, if necessary, continued only with close observation of the patient. Large bowel endoscopy has been recommended.

Antiperistaltic agents such as opiates and diphenoxylate with atropine (Lomotil) may prolong and/or worsen the condition. Vancomycin has been found to be effective in the treatment of antibiotic associated pseudomembranous colitis produced by *Clostridium difficile*. The usual adult dose is 500 milligrams to 2 grams of vancomycin orally per day in three to four divided doses administered for 7 to 10 days. Cholestyramine or colestipol resins bind vancomycin *in vitro*. If both a resin and vancomycin are to be administered concurrently, it may be advisable to separate the time of administration of each drug.

Diarrhea, colitis, and pseudomembranous colitis have been observed to begin up to several weeks following cessation of therapy with clindamycin.

#### INDICATIONS

Serious infections caused by susceptible anaerobic bacteria. Patients with serious infections due to susceptible strains of streptococci, pneumococci, and staphylococci in whom its use should be reserved for penicillin-allergic patients or other patients for whom, in the judgment of the physician, a penicillin is inappropriate.

Consider the nature of the infection and the suitability of less toxic alternatives (e.g., erythro-

mycin). Bacteriologic studies should be performed to determine the causative organisms and their susceptibility to clindamycin.

#### CONTRAINDICATIONS

History of hypersensitivity to clindamycin or lincomycin.

#### WARNINGS

See WARNING box. A toxin produced by *Clostridia* is one primary cause of antibiotic associated colitis. Cholestyramine and colestipol resins have been shown to bind the toxin *in vitro*. Mild cases of colitis may respond to drug discontinuance alone. Moderate to severe cases should be managed promptly with fluid, electrolyte and protein supplementation as indicated. Vancomycin has been found to be effective in the treatment of antibiotic associated pseudomembranous colitis produced by *Clostridium difficile*. The usual adult dosage is 500 mg to 2 grams of vancomycin orally per day in 3 or 4 divided doses for 7 to 10 days. Systemic corticoids and corticoid retention enemas may help relieve the colitis. Other causes of colitis should also be considered.

A careful inquiry should be made concerning previous sensitivities to drugs and other allergens. Because antagonism has been demonstrated between clindamycin and erythromycin *in vitro*, these drugs should not be administered concurrently. *Usage in Pregnancy:* Safety has not been established. *Usage in Newborns and Infants:* Appropriate monitoring of organ system functions is desirable. *Nursing Mothers:* Clindamycin has been reported to appear in breast milk in ranges of 0.7 to 3.8 mcg/ml. *Usage in Meningitis:* Since clindamycin does not diffuse adequately into the cerebrospinal fluid, it should not be used to treat meningitis.

**SERIOUS ANAPHYLACTOID REACTIONS REQUIRE IMMEDIATE EMERGENCY TREATMENT WITH EPINEPHRINE. OXYGEN AND INTRAVENOUS CORTICOSTEROIDS SHOULD ALSO BE ADMINISTERED AS INDICATED.**

#### PRECAUTIONS

Older patients with associated severe illness may tolerate diarrhea less well. When clindamycin is indicated in these patients, they should be carefully monitored for change in bowel frequency. Prescribe with caution in individuals with a history of gastrointestinal disease, particularly colitis and also in atopic individuals. Indicated surgical procedures should be performed in conjunction with therapy. Patients with severe renal disease and/or very severe hepatic disease accompanied by severe metabolic aberrations should be dosed with caution and serum clindamycin levels monitored during high dose therapy.

During prolonged therapy, periodic liver and kidney function tests and blood counts should be

performed. Use may result in overgrowth of non-susceptible organisms, particularly yeasts. Clindamycin has neuromuscular blocking properties and may enhance other neuromuscular blocking agents. Use with caution in patients receiving such agents. Do not inject clindamycin IV undiluted as a bolus. Dilute prior to IV administration to 300 mg per 50 ml or more of diluent. Infuse over at least 10-60 minutes. (See Dosage and Administration.) CLEOCIN HCl Capsules contain FD&C Yellow No. 5 (tartrazine) which may cause allergic-type reactions (including bronchial asthma) in certain susceptible individuals, especially in patients who also have aspirin hypersensitivity.

#### ADVERSE REACTIONS

**Gastrointestinal:** Abdominal pain, nausea, vomiting and diarrhea. (See WARNING box.)

**Hypersensitivity Reactions:** Maculopapular rash and urticaria. Generalized mild to moderate morbilliform-like skin rashes are the most frequent adverse reactions. Rare instances of erythema multiforme, some resembling Stevens-Johnson syndrome, have been reported. A few cases of anaphylactoid reactions have been reported. If a hypersensitivity reaction occurs, the drug should be discontinued. The usual agents should be available for emergency treatment. **Liver:** Jaundice and abnormalities in liver function tests have been observed. **Hematopoietic:** Neutropenia, eosinophilia, agranulocytosis and thrombocytopenia have been reported; no direct etiologic relationship to concurrent clindamycin therapy has been made. **Local Reactions:** Pain, induration and sterile abscess have been reported after intramuscular injection and thrombophlebitis after intravenous infusion. Reactions can be minimized or avoided by giving deep intramuscular injections and avoiding prolonged use of indwelling intravenous catheters. **Musculoskeletal:** Rare instances of polyarthritides have been reported. **Cardiovascular:** Rare instances of cardiopulmonary arrest and hypotension have been reported following too rapid IV infusion. (See Dosage and Administration.) **Renal:** Renal dysfunction has rarely been observed. No direct relationship has been established.

#### HOW SUPPLIED

Available as sterile solution with each ml containing clindamycin phosphate equivalent to 150 mg clindamycin base. Vials of 2 ml, 4 ml, and 6 ml.

CLEOCIN HCl as 75 mg and 150 mg capsules. Caution: Federal law prohibits dispensing without prescription.

For additional product information see your Upjohn representative.

J-6137

B-11-S

January 1986



A Century  
of Caring  
1886-1986

STANDARD OF EFFICACY WHENEVER ANAEROBES ARE SUSPECTED

**Cleocin Phosphate® 900 mg q 8h**  
(clindamycin phosphate injection) STERILE SOLUTION



# An established standard of efficacy

in serious pelvic infection

- Clinically effective against *Bacteroides fragilis* and other anaerobes commonly found in polymicrobial infections.
- Clinically effective against many gram-positive aerobes (eg, *Staphylococcus aureus*, group B streptococci) encountered in polymicrobial infections.

Clindamycin has been associated with *Clostridium difficile* colitis as have many other antibiotics (eg, cephalosporins, penicillins, and ampicillin). See Warnings in summary of prescribing information on the adjacent page.

**Cleocin  
Phosphate** STERILE  
SOLUTION  
(clindamycin phosphate injection)

900 mg q8h

Postcesarean endomyometritis  
(artist's interpretation)



A Century  
of Caring  
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## ORAL CONTRACEPTIVE (O.C.) AGENTS

**Indications:** Prevention of pregnancy. **DOSE-RELATED RISK OF THROMBOEMBOLISM.** Because studies have shown a positive association between O.C. dose and risk of thromboembolism, it is prudent to minimize estrogen exposure. Prescribe an O.C. with the least amount of estrogen compatible with an acceptable pregnancy rate and patient acceptance. Start new users on O.Cs containing 0.05 mg or less of estrogen.

**Contraindications** 1. Known or suspected pregnancy (see Warning #5). 2. Thrombophlebitis or thromboembolic disorders. 3. Past history of deep vein thrombophlebitis or thromboembolic disorders. 4. Undiagnosed abnormal genital bleeding. 5. O.Cs should not be used by women who have or have had any of the following: a. cerebral vascular or coronary artery disease, including myocardial infarction, b. known or suspected carcinoma of the breast, c. known or suspected estrogen dependent neoplasia, d. benign or malignant liver tumor that developed during use of O.Cs or other estrogen containing products.

**WARNINGS:** Cigarette smoking increases the risk of serious cardiovascular side effects from O.C. use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use O.Cs should be strongly advised not to smoke.

The use of O.Cs is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, liver tumor, gall bladder disease, visual disturbances, fetal abnormalities, and hypertension. Practitioners prescribing O.Cs should be familiar with the following information relating to these risks:

1. **Thromboembolic Disorders and Other Vascular Problems.** An increased risk of thromboembolic and thrombotic disease associated with O.C. use is established. One study demonstrated an increased relative risk for fatal venous thromboembolism and several studies demonstrated it for non-fatal venous thromboembolism. They estimate that O.C. users are 4-11 times more likely than nonusers to develop these diseases without evident cause. One British study reported an excess death rate of 40% in O.C. users, most of which resulted from cardiovascular disease. Another British study showed a lower death rate in O.C. users than controls; an increase in cardiovascular deaths was seen but was not statistically significant. A U.S. prospective study failed to disclose increased mortality rates from cardiovascular disorders, but a subset analyzed as a retrospective, case-control study showed significant increases in venous thromboembolism, CERE-BROVASCULAR DISORDERS. Two American studies demonstrated an increased relative risk for stroke not shown in prior British studies. In an American study of cerebrovascular disorders in women with and without predisposing causes, relative risk of hemorrhagic stroke was estimated as 2.0 times greater and thrombotic stroke as 4.9 times greater in users than nonusers. A British long-term follow-up study reported in 1979 a highly significant association between O.C. use and stroke. Another study had suggested an association in 1974, but the number of cases was too small to estimate the risk. Subarachnoid hemorrhage has been shown to be increased by O.C. use in British and American studies. Smoking alone increases incidence of these accidents; smoking and pill use appear to increase risk more than either alone.

**MYOCARDIAL INFARCTION (MI).** Increased relative risk of MI associated with O.C. use has been reported. One British study found that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of pre-eclamptic toxemia) the higher the risk of developing MI, regardless of O.C. use. O.Cs were an additional risk factor. In terms of relative risk, it has been estimated that nonsmoking O.C. users (smoking is considered a major predisposing condition to MI) are twice as likely to have a fatal MI as nonsmoking nonusers. O.C. users who are smokers have a 5-fold increased risk of fatal infarction compared to non-smoking users, and a 10-12 fold increased risk compared to nonsmoking nonusers. The number of cigarettes smoked is important. In determining the importance of these relative risks, baseline rates for various age groups must be considered. (Estimates are based on British vital statistics which show acute MI death rates 2-3 times less than in the U.S. so U.S. death rates could be higher.) Importance of other predisposing conditions in determining relative and absolute risks has not been quantified; other synergistic actions may exist. **RISK OF DOSE.** Using data from several national adverse reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to estrogen dose in O.Cs. O.Cs with 0.1 mg or more of estrogen were associated with a higher risk of thromboembolism than those containing 0.05-0.08 mg but quantity of estrogen may not be the sole factor. This was supported by a U.S. study. A British study found a positive association between dose of progestogen or estrogen and certain thromboembolic conditions. Swedish authorities noted decreased reporting of thromboembolic episodes when higher estrogen preparations were no longer prescribed. Careful epidemiological studies to determine degree of thromboembolic disease risk associated with progestogen-only O.Cs have not been done. Thromboembolic disease has been reported in women using these products, and they should not be considered free of access risk. **PERSISTENCE OF RISK.** Two studies have suggested an increased risk may persist for 6 years after discontinuation of O.C. use for cerebrovascular disease and 9 years for MI; another study suggested the persistence of risk for subarachnoid hemorrhage. **ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES.** A large British prospective study estimated mortality rate per 100,000 women per year from circulatory system diseases for O.C. users and nonusers according to age, smoking habits, and duration of use. The overall annual excess death rate for O.C. users was estimated to be 20/100,000 (ages 15-34 = 10/100,000; ages 35-44 = 33/100,000; ages 45-49 = 140/100,000). Risk is concentrated in long-term users and in smokers, and may persist after O.C. discontinuation. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all occurred in women 35 or older. An update provided the following rates: ages 15-34 = 16/1000 for nonusers and 1/2000 for smokers; ages 45 and over = 1/2500 for nonusers and 1/500 for smokers. Risk appeared to increase with parity, but not with duration of use. Until more women under 35 with continuing use for 5 or more years are available, it is not possible to assess relative risk for this age group. Data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. Estimates include combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease for O.Cs) plus risk attributable to noncontraceptive method (e.g., myocardial infarction). The relative risk of death associated with the contraceptive method is shown in the following table. The study concluded that mortality associated with all contraceptive methods is below that of childbirth, except for O.Cs in women over 40 who smoke. (Rates given for pill only/smokers for each age are for smokers as a class. For "heavy" smokers (more than 15 cigarettes a day) the rates would be about double, for "light" smokers (less than 15), about half.) The lowest mortality is with the condom or diaphragm backed up by early abortion. The study also concluded that O.C. users who smoke, especially over 30, have greater mortality risk than O.C. users who do not smoke.

Risk of thromboembolic and thrombotic disease associated with O.Cs increases with age after 30 and for MI is further increased by hypertension, hyperlipidemia, obesity, diabetes, or history of pre-eclamptic toxemia, and especially by smoking. The following chart is a rough estimate of risk of death from circulatory disorders associated with O.C. use.

## SMOKING HABITS AND OTHER PREDISPOSING CONDITIONS—RISK ASSOCIATED WITH USE OF O.Cs

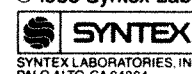
Age	Below 30	30-39	40 +
Heavy smokers	C	B	A
Light smokers	D	C	B
Non-smokers	(no predisposing conditions)	D	C
(other predisposing conditions)	D	C	B

A—Use associated with very high risk.  
B—Use associated with high risk.  
C—Use associated with moderate risk.  
D—Use associated with low risk.

Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, discontinue O.C. immediately. A 4-6 fold increased risk of post-surgery thromboembolic complications has been reported in O.C. users. If feasible, discontinue O.Cs at least 4 weeks before surgery associated with increased risk of thromboembolism or prolonged immobilization. Before resuming O.C. after major surgery or bedrest, balance risks of post-surgery thromboembolic complications with contraceptive needs. Data suggest variable venous stasis depending on the severity of the varicosities. 2. **Ocular Lesions:** Neuro-ocular lesions such as optic neuritis or retinal thrombosis have been associated with O.C. use. Discontinue O.C. if there is unexplained, sudden, or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema, or retinal vascular lesions; and institute appropriate diagnostic and therapeutic measures. 3. **Carcinoma:** Long-term continuous administration of natural or synthetic estrogen in certain animals increases certain tumors, benign or malignant, such as breast, cervix, vagina, uterus, ovary, pituitary and liver. Certain synthetic progestogens, now currently in O.Cs, increase the incidence of mammary nodules, benign and malignant, in dogs. Several retrospective case-control studies reported an increased relative risk (3.1-13.9 times) associating endometrial carcinoma with the prolonged use of estrogens in postmenopausal women. One publication reported the first 30 cases submitted to the registry of cases of adenocarcinoma of the endometrium in women under 40 on O.Cs. Of the adenocarcinomas found in women without predisposing risk factors for adenocarcinoma of the ovum (e.g., irregular bleeding when O.Cs were first given, polycystic ovaries); nearly all occurred in women who had used sequential O.Cs, which are no longer marketed. No statistical association has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only O.Cs, although individual cases have been reported. Studies have shown no increased risk of breast cancer to O.C. or estrogen users. One study found no overall increased risk of breast cancer in O.C. users but a greater risk was suggested for O.C. users with documented benign breast disease and for long-term users. Another study found a higher rate of breast cancer among grandmothers or aunts was significantly more frequent among breast cancer patients who had used an O.C. continuously for one or more years than among nonusers with breast cancer. One other study indicated an increased risk of breast cancer in women taking menopausal estrogens, which increased with duration of follow-up. One author suggests that extended (over 6 years) O.C. use prior to first full term pregnancy was associated with a significant relative risk of breast cancer. A reduced occurrence of benign breast tumors in O.C. users has been well documented. One study reported malignant melanoma more frequently in O.C. users than controls and suggests an increased incidence of urinary tract and thyroid cancers. A prospective study of women with cervical dysplasia found an increase in severity and conversion to cancer *in situ* in O.C. users compared to nonusers. This became statistically significant after 3-4 years of use. Non-invasive dysplasia in the cervix in 6 months of pill use was suggested to predict progression after prolonged exposure. One study disclosed an increased risk of cancer of the cervix (largely carcinoma *in situ*) in O.C. users under 40, particularly those who had used O.Cs over 4 years. There have been other reports of microglanular hyperplasia of the cervix in O.C. users. One study reported an association between O.C. use and endocervical adenocarcinoma. In summary, there is no confirmed evidence from human studies of increased risk of cancer associated with O.Cs. Close clinical surveillance of all O.C. users is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, take appropriate diagnostic measures to rule out malignancy. Monitor O.C. users with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms with particular care. 4. **Liver Tumors:** Sudden severe abdominal pain or shock may be due to rupture and hemorrhage of a liver tumor. There have been reports associating benign or malignant liver tumors with short-term and long-term O.C. use. One study reported use of O.Cs with high hormonal potency and age over 30 may further increase risk of hepatocellular adenoma. Two studies relate risk with duration of use, risk being much greater after 4 or more years of use. Long-term O.C. users have an estimated annual incidence of hepatocellular adenoma of 3-4/100,000. Although an uncommon lesion, it should be considered in women presenting with an "acute abdomen." The tumor may cause serious or fatal hemorrhage. Patients with liver tumors have demonstrated variable clinical features which may make preoperative diagnosis difficult. Some cases presented because of right upper quadrant masses, while most had signs and symptoms of acute intra-abdominal hemorrhage. The following are laboratory abnormalities which may be helpful: liver scans may show a focal defect; hepatic arteriography may be useful in diagnosing primary liver neoplasms. 5. **Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring:** Use of female sex hormones—estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. Females exposed *in utero* to diethylstilbestrol (DES), a synthetic estrogen, have an increased risk of developing in later life a form of vaginal cancer or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1/1000 exposures or less. Although there is no evidence that O.Cs further enhance the risk of developing this type of malignancy, such O.C. users should be monitored with particular care. A high percentage of women exposed to diethylstilbestrol (30-50%) have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are a precursor of vaginal malignancy. Male children so exposed may develop urogenital tract abnormalities. Although similar data are not available on other estrogens, it cannot be presumed that they would not induce similar changes. Increased risk of congenital anomalies, including heart and limb defects, has been reported following use of sex hormones including O.Cs, in pregnancy. One case-control study estimated a 4.7-fold increased relative risk of limb-reduction defects in infants exposed *in utero* to sex hormones (O.Cs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some exposures involved only a few days of treatment. Data suggest risk of limb-reduction defects in exposed fetuses is somewhat less than 1/1000 live births. In a large prospective study, cardiovascular disease, diabetes, and abnormal laboratory values were reported in children of women who used O.Cs during early pregnancy. Increased female hormones, including O.Cs, during early pregnancy occurred at 18-21 months, compared to 78/1000 for children not so exposed *in utero*. These results are statistically significant. A Welsh study found a statistically significant excess of neural tube defects among offspring of prior O.C. users (within 3 months) than among controls. The incidence of two births may be increased for women who conceived within 3 months after stopping O.C. use. In the past, female sex hormones were used during pregnancy in an attempt to treat threatened or habitual abortion. There is evidence that estrogens are ineffective, and there is no evidence from well controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing O.C. use. If pregnancy is confirmed, tell the patient about potential risks to the fetus and discuss advisability of continuing the pregnancy. Women who discontinue O.Cs to become pregnant should use an alternate form of contraception for a period of time before attempting to conceive. A 1-month period is suggested by a study suggesting increased frequency

of neural tube defects in women impregnated during the first 3 months after cessation of O.C. use. Do not use progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding as a pregnancy test. 6. **Gall Bladder Disease:** Studies report increased risk of gall bladder disease in O.C. or estrogen users. In one study, an increased risk appeared after 2 years of use and doubled after 4-5 years. In another study, an increased risk was apparent between 6 and 12 months. 7. **Carbohydrate and Lipid Metabolic Effects:** Because a decrease in glucose tolerance has been observed in a significant percentage of patients on O.Cs, prediabetic and diabetic O.C. users should be carefully observed. An increase in triglycerides and total phospholipids has been observed in O.C. users but its clinical significance is unknown. 8. **Elevated Blood Pressure:** An increase in blood pressure has been reported with O.C. use. Hypertension may occur within a few months of beginning O.Cs. In the first year of use, incidence of hypertension may be no higher in O.C. users than in nonusers. Incidence in users increases with exposure and in the fifth year of use is 2-3 times that in the first year. Age is strongly correlated with hypertension in O.C. users. Women with a history of elevated blood pressure (hypertension), preexisting renal disease, history of toxemia or elevated blood pressure during pregnancy, familial tendency to hypertension or its consequences, or history of excessive weight gain or fluid retention during the menstrual cycle may be more likely to develop elevated blood pressure when given O.Cs and should be monitored closely. Even though elevated blood pressure may remain within the "normal" range, closely watch elevations, particularly for women with other risk factors for cardiovascular disease or stroke. High blood pressure may or may not persist after O.C. discontinuation. 9. **Headache:** Discontinue O.C. and evaluate the cause of onset or exacerbation of migraine or development of a new pattern of headache which is recurrent, persistent, or severe. 10. **Bleeding Irregularities:** Breakthrough bleeding, spotting, and missed menses often make users discontinue O.Cs. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, consider nonfunctional causes. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, use adequate diagnostic measures to rule out pregnancy or malignancy. If pathology has been excluded, time or another formulation may solve the problem. While potentially useful in minimizing menstrual irregularity, change to an O.C. with a higher estrogen content only if necessary since this may increase the risk of thromboembolic disease. Women with a history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may tend to remain anovulatory or to become amenorrheic after O.C. discontinuation. Women with these preexisting problems should be advised of this and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may occur in women without previous irregularities. A higher incidence of galactorrhea and of pituitary tumors (e.g., adenomas) has been associated with amenorrhea in former users compared with nonusers. One study reported a 16-fold increased incidence of pituitary prolactin-secreting tumors among patients with postpill amenorrhea who had galactorrhea was present. 11. **Fertility:** There is evidence of impairment of fertility in women discontinuing O.Cs in comparison with other contraceptive methods, which appears to be independent of duration of use. While impairment diminishes with time, there is an appreciable difference in the results in nulliparous women for O.C. and other groups 30 months after discontinuation of birth control. For parous women the difference is not apparent 30 months after discontinuation of contraception. 12. **Ectopic Pregnancy:** Ectopic as well as intrauterine pregnancy may occur in O.C. failure. In progestogen-only O.C. failures, the ratio of ectopic to intrauterine pregnancies is higher than in nonusers, since the drugs are more effective in preventing intrauterine than ectopic pregnancies. 13. **Breast Feeding:** O.Cs in the postpartum period may interfere with lactation by decreasing quantity and quality of breast milk. A small fraction of O.C. components may be present in milk of mothers receiving O.Cs. Effects, if any, on the breast-fed child have not been determined. If feasible, defer O.C. use until the infant has been weaned. **Precautions GENERAL 1.** Take a complete medical and family history before starting O.Cs. Pretreatment and periodic physical exams should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papapanicolaou smear and relevant lab tests. Examine O.Cs should not be prescribed for longer than 1 year without another physical examination. 2. **Estrogen:** Estrogen-progestogen preparations, preexisting uterine leiomyomata may enlarge. 3. **Observe patients with a history of psychic depression and discontinue O.Cs if depression recurs to a serious degree.** Patients becoming significantly depressed while taking O.Cs should stop the O.C. and use an alternate method of contraception to determine whether the symptom is drug related. 4. **O.Cs may impair judgment and motor function.** Prescribe with caution, and only with careful monitoring, in patients with conditions that might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma or cardiac, hepatic or renal insufficiency. 5. **Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while using O.Cs.** Discontinue O.C. if jaundice develops. 6. **Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients.** 7. **O.C. users may have disturbances in normal tripartite metabolism that may result in a relative pyridoxine deficiency.** The clinical significance is unknown. 8. **Serum folate levels may be depressed by O.C. use.** Since the pregnant woman is predisposed to folate deficiency and incidence of folate deficiency increases with increasing gestation, if a woman becomes pregnant shortly after stopping O.Cs, she has a greater chance of developing folate deficiency and related complications. 9. **Advise the pathologist of O.C. use when relevant specimens are submitted.** 10. **Certain endocrine and liver function tests and blood components may be affected by estrogen-containing O.Cs.** For example: a. increased subcutaneous fat retention, b. increased prothrombin and factors VII, VIII, IX, and X, decreased antithrombin, c. increased norepinephrine-induced platelet aggregability, d. increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered, d. decreased pregnenolone excretion, e. reduced response to metoprolol test, f. increased phospholipids and triglycerides, g. temporarily decreased glucose tolerance. 11. **Patients with conditions that develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist and temporary or permanent cessation of wear considered.** 12. **DRUG INTERACTIONS:** O.Cs may be less effective and there may be increased breakthrough bleeding from interactions with rifampin, isoniazid, ampicillin, tetracycline, neomycin, penicillin V, chloramphenicol, sulfonamides, nitrofurantoin, barbiturates, phenytoin, primidone, analgesics, tranquilizers, antipsychotics, antiepileptics, antihypertensives, O.Cs may alter effectiveness of such other drugs as oral anticoagulants, anticonvulsants, tricyclic antidepressants, antihypertensives (e.g., guanethidine), vitamins, hypoglycemic agents, tranquilizers, hypnotic preparations, theophylline, and beta blockers. **CARCINOGENESIS** See Warnings. **PREGNANCY** Pregnancy category X. See Contraindications and Warnings. **NURSING MOTHERS:** See Warnings. **Adverse Reactions:** An increased risk of the following serious adverse reactions has been associated with O.C. use (see Warnings): thrombophlebitis, thrombosis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, mesenteric thrombosis, liver tumors, cerebral hemorrhage, hypertension, gall bladder disease, congenital anomalies, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis, Raynaud's disease, arterial thromboembolism. The following adverse reactions have been reported in O.C. users and are believed to be drug related: bleeding irregularities (breakthrough bleeding, spotting, missed menses during treatment, amenorrhea after treatment), gastrointestinal symptoms (nausea, vomiting, bloating, abdominal cramps, colitis), dysmenorrhea, infertility after discontinuance of treatment, edema, chloasma or melasma which may persist after drug is discontinued, breast changes (tenderness, enlargement, and secretion), intolerance to contact lenses, changes in corneal curvature (steepening), change in weight (increase or decrease), change in cervical erosion and cervical secretion, possible diminution of lactation when given immediately postpartum, cholestatic jaundice, migraine, increase in size of uterine leiomyomata, rash (allergic), mental depression, reduced tolerance to carbohydrates, vaginal candidiasis, prolactin-secreting pituitary tumors, chills. The following adverse reactions have been reported in O.C. users and the association has been neither confirmed nor refuted: premenstrual-like syndrome, catarracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of hair, erythema multiforme, erythema nodosum, hemorrhagic eruptions, vaginitis, porphyria, impaired renal function, malignant nephrosclerosis (hemolytic uremic syndrome), malignant hypertension, acute renal failure. **Information for the Patient** (See Patient Package Insert).

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Serious as well as minor side effects have been reported following the use of all oral contraceptives. These include thromboembolic disease. Please see brief summary of full prescribing information on next page.

1. 1/35 formulations contain 1.0 mg norethindrone with 0.035 mg ethinyl estradiol. BTB comparisons are based on the first three cycles of use. Data available from Syntex Laboratories, Inc.
2. Wynn V, Niththyananthan R: The effect of progestins in combined oral contraceptives on serum lipids with special reference to high-density lipoproteins. *Am J Obstet Gynecol* 142:766-772, 1982.
3. Wynn V: Effect of duration of low-dose oral contraceptive administration on carbohydrate metabolism. *Am J Obstet Gynecol* 142:739-746, 1982.
4. In an independent survey the Walleto™ pill dispenser was preferred to the Ortho Dialpak by 7 out of 10 prospective OC patients. Data available from Syntex Laboratories, Inc.



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†Serious as well as minor side effects have been reported with the use of oral contraceptives. The physician should remain alert to the earliest manifestations of any symptoms of serious disease and discontinue oral contraceptive therapy when appropriate. Please see complete Prescribing Information, a summary of which appears on the preceding page.

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**IMPORTANT NOTE—This information is a BRIEF SUMMARY of the complete prescribing information provided with the product and therefore should not be used as the basis for prescribing the product. This summary was prepared by deleting from the complete prescribing information certain text, tables, and references. The physician should be thoroughly familiar with the complete prescribing information before prescribing the product.**

### INDICATIONS AND USAGE: PREVENTION OF PREGNANCY.

**DOSE-RELATED RISK OF THROMBOEMBOLISM FROM ORAL CONTRACEPTIVES:** Two studies have shown a positive association between the dose of estrogens in oral contraceptives and the risk of thromboembolism. For this reason, it is prudent and in keeping with good principles of therapeutics to minimize exposure to estrogen. The oral contraceptive product prescribed for any given patient should be that product which contains the least amount of estrogen that is compatible with an acceptable pregnancy rate and patient acceptance. It is recommended that new acceptors of oral contraceptives be started on preparations containing 0.05 mg or less of estrogen.

**CONTRAINDICATIONS:** Oral contraceptives should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophlebitis or thromboembolic disorders. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Oral contraceptive tablets may cause fetal harm when administered to a pregnant woman. Oral contraceptive tablets are contraindicated in women who are pregnant. If the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus (see WARNINGS, No. 5). 8. Benign or malignant liver tumor which developed during the use of oral contraceptives or other estrogen-containing products.

### WARNINGS

**Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke. The use of oral contraceptives is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.**

1. **THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS.** An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Four principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of oral contraceptives are 4 to 10 times more likely than nonusers to develop these diseases without evident cause. Overall excess mortality due to pulmonary embolism or stroke is on the order of 10 to 35 deaths annually per 100,000 users and increases with age. **CEREBROVASCULAR DISORDERS.** In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than in nonusers and the risk of thrombotic stroke was 4.0 to 9.5 times greater in users than in nonusers. A prospective study conducted in Great Britain estimated that former users have a risk for all cerebrovascular disease 2.6 times greater than that of nonusers. This risk remained elevated for at least six years after last oral contraceptive use. A prospective study conducted in the United States found that past use of oral contraceptives was associated with increased risk of subarachnoid hemorrhage, the relative risk being 5.3. There was also some evidence from this study that the degree of risk may be related to duration of oral contraceptive use. **MYOCARDIAL INFARCTION:** An increased risk of myocardial infarction associated with the use of oral contraceptives has been reported confirming a previously suspected association. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of premyocardial infarction, etc.), the higher the risk of developing myocardial infarction, regardless of whether the patient was an oral contraceptive user or not. Oral contraceptives, however, were found to be a clear additional risk factor. The annual excess case rate (increased risk) of myocardial infarction (fatal and nonfatal) in oral contraceptive users was estimated to be approximately 7 cases per 100,000 women users in the 30-39 age group and 67 cases per 100,000 women users in the 40-44 age group. In terms of relative risk, it has been estimated that oral contraceptive users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. Oral contraceptive users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified, it is quite likely that the same synergistic action exists, but perhaps to a lesser extent. A study suggests that some increased risk of myocardial infarction in oral contraceptive users persists following discontinuation of oral contraceptives and that the degree of the residual risk is related to the duration of the past use. **Risk of Dose:** In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in oral contraceptives. Preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the United States. Careful epidemiological studies to determine the degree of thromboembolic risk associated with progestogen-only oral contraceptives have not been performed. Cases of thromboembolic disease have been reported in women using these products, and they should not be presumed to be free of excess risk. The risk of thromboembolic and thrombotic disorders, in both users and nonusers of oral contraceptives, increases with age. Oral contraceptives are, however, an independent risk factor for these events. **ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES:** A large prospective study carried out in the United Kingdom estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of oral contraceptives according to age, smoking habits, and duration of use. The overall excess death rate annually from circulatory diseases for oral contraceptive users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), the risk being concentrated in older women, in those with a long duration of use, and in cigarette smokers. It was not possible, however, to examine the interrelationships of age, smoking, and duration of use, nor to compare the effects of continuous versus intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for five or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for five or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. This study reports that the increased risk of circulatory disease mortality may persist after the pill is discontinued. Another study published at the same time confirms a previously reported increase of mortality in pill users from cardiovascular disease. The study concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of oral contraceptives in women over 40 who smoke. (The rates given for pill only smokers for each age group are for smokers as a class, for "heavy" smokers [more than 15 cigarettes a day], the rates given would be about double, for "light" smokers [less than 15 cigarettes a day], about 50 percent.) The mortality associated with oral contraceptive use in nonsmokers over 40 is higher than with any other method of contraception in that age group. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with oral contraceptives increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of premyocardial infarction and especially by cigarette smoking. The risk of myocardial infarction in oral contraceptive users is substantially increased in women age 40 and over, especially those with other risk factors. The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four- to six-fold increased risk of postsurgery thromboembolic complications has been reported in oral contraceptive users; if feasible, oral contraceptives should be discontinued at least four weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization. 2. **OCULAR LESIONS.** There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of oral contraceptives. Discontinue oral contraceptive medication if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions and institute appropriate diagnostic and therapeutic measures. 3. **CARCINOMA.** Long-term continuous administration of either natural or synthetic estrogens in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in oral contraceptives, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case-control studies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on oral contraceptives. Of the cases found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time oral contraceptives were first given, polycystic ovaries), nearly all occurred in women who had used a sequential oral contraceptive. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only oral contraceptives. Several studies have found no increases in breast cancer in women taking oral contraceptives or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with oral contraceptives, found an excess risk in the subgroups of oral contraceptive users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of oral contraceptives has been well-documented. In summary there is at present no confirmed evidence from human studies of an increased risk of cancer associated with oral contraceptives. Close clinical surveillance of all women taking oral contraceptives is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. 4. **HEPATIC TUMORS.** Benign hepatic adenomas have been found to be associated with the use of oral contraceptives. One study showed that oral

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## Tablets

contraceptive formulations with high hormonal potency were associated with a higher risk than lower potency formulations and use of oral contraceptives with high hormonal potency and age over 30 years may further increase the woman's risk of hepatocellular adenoma. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of oral contraceptives. Two studies relate risk with duration of use of the contraceptive, the risk being much greater after four or more years of oral contraceptive use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women taking oral contraceptives. The relationship of these drugs to the type of malignancy is not known at this time. 5. **USE IN OR IMMEDIATELY PRECEDING PREGNANCY BIRTH DEFECTS IN OFFSPRING, AND MALIGNANCY IN FEMALE OFFSPRING.** The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be on the order of 1 to 4 in 1000 exposures. Although there is no evidence at the present time that oral contraceptives further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. Furthermore, a high percentage of sub-exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including oral contraceptives, in pregnancy. One case control study has estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortions from women who become pregnant soon after ceasing oral contraceptives. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping oral contraceptives is unknown. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period (or after 45 days from the last menstrual period if the progestogen-only oral contraceptives are used), and further use of oral contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue oral contraceptives with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend three months although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy. 6. **GALLBLADDER DISEASE.** Studies report an increased risk of surgically confirmed gallbladder disease in users of oral contraceptives and estrogens. In one study, an increased risk appeared after two years of use and doubled after four or five years of use. In one of the other studies, an increased risk was apparent between six and twelve months of use. 7. **CARBONYDRATE AND LIPID METABOLIC EFFECTS.** A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. For this reason, prediabetic and diabetic patients should be carefully observed while receiving oral contraceptives. An increase in triglycerides and total phospholipids has been observed in patients receiving oral contraceptives. The clinical significance of this finding remains to be defined. 8. **ELEVATED BLOOD PRESSURE.** An increase in blood pressure has been reported in patients receiving oral contraceptives. In some women hypertension may occur within a few months of beginning oral contraceptive use. In the first year of use, the prevalence in users increases, however, with longer exposure, and in the fifth year of use is two and a half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in oral contraceptive users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given oral contraceptives. Hypertension that develops as a result of taking oral contraceptives usually returns to normal after discontinuing the drug. 9. **HEADACHE.** The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of oral contraceptives and evaluation of the cause. 10. **BLEEDING (IRREGULARITIES).** Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to another formulation with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of oral contraceptives. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Postuse anovulation, possibly prolonged, may also occur in women without previous irregularities. 11. **ECTOPIC PREGNANCY.** Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. 12. **BREAST FEEDING.** Oral contraceptives given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The effects, if any, of the breast-fed child have not been determined. If feasible, the use of oral contraceptives should be deferred until the infant has been weaned. **PRECAUTIONS:** General: 1. A complete medical and physical examination should be taken prior to the initiation of oral contraceptives. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, oral contraceptives should not be prescribed for longer than one year without another physical examination being performed. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking oral contraceptives should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. 4. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving oral contraceptive therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. Oral contraceptive users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by oral contraceptive therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping oral contraceptives, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of oral contraceptive therapy when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing oral contraceptives: a. Increased sulfobromophthalen retention; b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin; c. increased norepinephrine-induced platelet aggregability; c. increased thyroid-binding globulin (TBG) leading to increased circulating thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion; e. Reduced response to metoprolol test. **INFORMATION FOR THE PATIENT: (See Patient Package Insert).** **DRUG INTERACTIONS:** Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin, griseofulvin, and tetracycline. **CARCINOGENESIS, PREGNANCY, NURSING MOTHERS: See CONTRAINDICATIONS AND WARNINGS. ADVERSE REACTIONS:** An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS): Thrombophlebitis, Pulmonary embolism, Coronary thrombosis, Cerebral thrombosis, Cerebral hemorrhage, Hypertension, Gallbladder disease, Liver tumors. Congenital anomalies. There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis, Neuro-ocular lesions, e.g., optic neuritis, and optic neuritis. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea, usually the most common adverse reaction. Vomiting occurs in approximately 10% or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding, Spotting. Change in menstrual flow. Dysmenorrhea. Amenorrhea during and after treatment. Temporary infertility after discontinuance of treatment. Edema. Chloasma or melasma which may persist. Breast changes: tenderness, enlargement, and secretion. Change in weight (increase or decrease). Change in cervical erosion and cervical secretions. Possible diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine. Increase in size of uterine leiomyomata. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives, and the association has been neither confirmed nor refuted: Premenstrual-like syndrome. Cataracts. Changes in libido. Chorea. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiforme. Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. Hemolytic uremic syndrome. **OVERDOSAGE:** Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

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# The effects of oral administration of progesterone for premature labor

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The potential tocolytic effect of natural progesterone administration on premature labor was investigated in a double-blind study. An oral progesterone formulation was used because its ability to increase both plasma and myometrial concentration of progesterone in pregnant women had been previously demonstrated. Furthermore, no commercial intravenous or intramuscular natural progesterone formulation is currently available in France. Fifty-seven patients in two obstetric clinics, admitted because of the risk of premature delivery, were included in the study, and uterine contractility and fetal cardiac rhythm were monitored in all of them. At random and after 30 minutes' rest, 29 women absorbed four capsules of 100 mg of progesterone each and 28 women absorbed four capsules of a placebo. Plasma progesterone levels were evaluated in all cases after 30 minutes' rest and 1 hour after absorption of the capsules. The results showed that bed rest and placebo administration decrease uterine activity in 42% of the cases and oral progesterone decreases activity in 75% to 88% of cases, depending on the initial severity of the menace of premature delivery. The difference between the effects of progesterone and of placebo is significant. The tocolytic effect of oral progesterone is not as intense or as rapid as the effect of intravenous  $\beta$ -mimetics but is sufficient in 80% of cases, on the average, to stop the premature labor without any detectable side effects. This tocolytic effect of oral progesterone is related not just to an increase in plasma progesterone levels but probably to an increase in myometrial progesterone concentration. (AM J OBSTET GYNECOL 1986;154:525-9.)

**Key words:** Premature labor, tocolytic drugs, progesterone

Progesterone is of great usefulness in permitting pregnancy to reach its physiologic term, since it inhibits uterine contractility. At sufficient levels in the myometrium, it blocks the oxytocin effect<sup>1,2</sup> of  $F_{2\alpha}$ <sup>3</sup> and  $\alpha$ -adrenergic stimulation and therefore increases the  $\beta$ -adrenergic tocolytic response.<sup>4,5</sup> The mechanisms of these progesterone tocolytic effects, in relation to the principal myometrial contractility stimulating agents, have been identified in various animal species. Estradiol increases the concentration of oxytocin receptors while progesterone decreases it.<sup>1</sup> Estradiol increases the local synthesis of prostaglandin  $F_{2\alpha}$ ; progesterone decreases it.<sup>6</sup> In addition, the latter neutralizes myometrial sensitivity to the exogenous perfusion of prostaglandin  $F_{2\alpha}$ .<sup>2</sup> Estradiol increases the  $\alpha$ -adrenergic uterine receptors whereas progesterone decreases them,<sup>5,7</sup> thus enhancing the  $\beta$ -adrenergic response. Finally, sufficient levels of progesterone modify the ultrastructural organization of the myometrium by inhibiting the appearance of intercellular gap junctions. Thus proges-

terone prevents myometrial organization of syncytium, which is capable of propagating electrical stimulations and responding with a coordinated muscular contraction.<sup>8</sup>

Natural progesterone, produced spontaneously in great abundance during normal pregnancy, is devoid of any disturbing teratogenic, metabolic, or hemodynamic effects. This is contrary to certain artificial progestagens and  $\beta$ -mimetics.<sup>9</sup>

Several attempts at treating premature labor have already been carried out with molecules considered to be similar to progesterone, such as chlormadinone acetate or 17-hydroxyprogesterone caproate. However, the fact that these steroids are considerably different from natural progesterone may decrease their binding to myometrial progesterone receptors<sup>3</sup> and thus their efficacy. Furthermore, the studies published to date have not been adequately controlled and therefore have not produced convincing results (see Reference 18). For these reasons we considered it of interest to test the major therapeutic advantages of natural progesterone in scientifically and ethically acceptable study conditions.

## Methods

This study was made up of 57 women, all admitted between the thirtieth and thirty-sixth week of amen-

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orrhea for risk of preterm delivery, who were patients in the obstetric unit of two different hospitals in Marseilles and Paris.

The following examinations were carried out.

1. On admission to the ward, clinical control was established by the noting of the frequency and intensity of uterine contractions, the state of the membranes and cervix, and the existence of metrorrhagia according to Baumgarten's score for premature labor.

2. During 30 minutes of bed rest, fetal cardiac rhythm and uterine contractility were monitored continuously.

3. After 30 minutes of monitoring, a new clinical examination was performed and blood taken for evaluation of progesterone plasma levels. Four capsules, each containing either 100 mg of micronized progesterone (Utrogestan) or a placebo (randomly selected and unknown to the patient and physician), were administered in a single oral dose.

4. During the hour following absorption of the capsules, clinical control and monitoring of cardiac rhythm and uterine contractility continued.

5. At the end of the hour of observation a new blood test for evaluation of plasma progesterone was performed.

6. In a first group of 20 patients (at Hôpital St. Antoine in Paris) the control of uterine contractility was continued, as required, by usual intravenous  $\beta$ -mimetics. In a second group of 37 patients (at Hôpital de la Conception in Marseilles) the study of the tocolytic effects of oral progesterone was prolonged as follows: During the hour following absorption of the four capsules (progesterone or placebo) and when the tocolytic effect was judged sufficient, clinical observation and electric monitoring were continued and four capsules of oral progesterone were administered every 4 to 8 hours until the patient's discharge from the hospital. However, when the tocolytic effect was insufficient, administration of intravenous  $\beta$ -mimetics (ritodrine) was begun immediately.

7. Progesterone was evaluated in all of the blood samples by radioimmunoassay at the same time and by the same laboratory (Centre Hospitalo-Universitaire La Pitié, Paris, France).

## Results

The evolution of the frequency of contractions during the observation period, assessed on clinical criteria and on graphic recordings, was noted in writing before revealing the contents (progesterone or placebo) of the capsules administered.

In the first group of 20 patients (in Paris) the frequency of contractions remained identical or increased in nine patients, seven of whom received the placebo and the other two the progesterone. The frequency declined in 11 patients, three of whom received the

placebo and eight the progesterone. Thus an improvement was noted in 30% of cases 1 hour after ingestion of the placebo and in 80% of cases 1 hour after ingestion of progesterone.

In the second group of 37 patients (in Marseilles) the frequency of the contractions remained identical or increased in 14 patients, nine of whom received the placebo and five the progesterone. The frequency declined in 23 patients, nine of whom received the placebo and 14 the progesterone. Thus an improvement was noted in 50% of cases 1 hour after ingestion of the placebo and in 73% of cases 1 hour after progesterone.

In these two groups, a total of 57 patients, the frequency of contractions decreased by 42.8% in those receiving four capsules of the placebo and by 75.8% in those receiving 400 mg of progesterone.

In these 57 patients the frequency of contractions went from a mean of 3.67 per 10 minutes (ranging from 1.5 to 7) to 2.91 (ranging from 0 to 9) during the hour of observation with the placebo recipients (28 cases) and to 1.93 (ranging from 0 to 4) during the hour of observation with the progesterone recipients (29 cases). The frequency of contractions decreased significantly ( $p < 0.001$ ) in the group treated with progesterone but in a nonsignificant manner in the group given the placebo ( $0.3 > p > 0.05$ ).

In seven cases the frequency did not alter during the hour following ingestion of 400 mg of progesterone. At the end of the hour of observation, administration of intravenous  $\beta$ -mimetics (Ritodrine) was immediately started.

This supplementary treatment coincided with a decrease in contractions in three cases, but in the other four cases (corresponding on admission to Baumgarten's score of 3 or 4 with ruptured membranes) tocolysis could not be obtained by the addition of intravenous  $\beta$ -mimetics. If we consider these four cases as inaccessible to medical treatment and exclude them from the study, the percentage of efficacy with oral progesterone use rises from a mean of 75.8% to 88% (and that of  $\beta$ -mimetics to 100%).

In the 16 cases in which the frequency of contractions remained unaltered in the hour following administration of the placebo, use of intravenous ritodrine permitted tocolysis each time. None of these cases presented with ruptured membranes.

In the second group of 37 patients (in Marseilles) the 23 patients in whom the contractions declined during the hour of observation, oral progesterone was continued up to the thirty-sixth week. The doses during the first 3 days were adjusted as required and according to the clinical evolution, with 400 mg every 4 to 8 hours. From the third day the dose was reduced to a mean of three daily doses of 200 mg. Delivery was delayed by a mean of 6.7 weeks (ranging from 2 to 14 weeks) and up to the thirty-eighth week of gestation (ranging from

**Table I.** Frequency of uterine contractions 1 hour after administration of placebo or oral progesterone (for risk of premature delivery, 57 cases)

	Increasing or steady frequency	Decreasing frequency	
		n	Total %
Placebo			
Group I	7	3	42.8
Group II	9	9	
Utrogestan			
Group I	2	8	75.8
Group II	5	14	

week 36 to week 40), with a mean birth weight of 3.07 kg (ranging from 2.02 to 3.90 kg). Only two birth weights were inferior to 2.5 kg (2.02 and 2.1 kg); they were in the cases of two primiparous women, aged 18 and 22, in whom premature labor had started during the weeks 30 and 34 with birth occurring during weeks 37 and 38, respectively. There were no unusual events during or after delivery, and in particular, neither hemorrhage or uterine inertia was observed.

Tolerance to oral progesterone in these circumstances was perfect except for a slight feeling of drowsiness in some cases during the period of acute treatment. Above all, no modifications in cardiac rhythm or blood pressure were noted. There was no nausea or digestive or respiratory disorders.

The plasma level of progesterone was a mean of 101.6 ng/ml (ranging from 44 to 265) on admission. It increased significantly ( $p < 0.05$ ) to 152.19 ng/ml (from 46 to 570 ng/ml) 1 hour after ingestion of 400 mg of progesterone and decreased nonsignificantly to 87.4 ng/ml (from 33 to 144 ng/ml) after ingestion of the placebo.

Irrespective of treatment (progesterone or placebo) in 34 cases with a decrease in the frequency of contractions, a mean increase in plasma progesterone of 29% ( $p < 0.05$ ) was observed. The absence of a clinical improvement in 23 other cases coincided with a slight nonsignificant decrease in progesterone plasma levels. However, a large minority of about 30% of the patients improved clinically despite the absence of increased progesterone plasma levels. Moreover, in the group of women who showed no clinical improvement, 35% had a sharp increase in plasma levels of progesterone.

#### Comment

Because of numerous undesirable maternal and fetal side effects it has been recently recommended to restrain as far as possible the use of  $\beta$ -mimetic drugs in premature labor.<sup>9</sup>

The tocolytic effects of progesterone are theoretically of great interest, since they inhibit the activity of

**Table II.** Frequency of uterine contractions 1 hour after administration of placebo or oral progesterone (for risk of premature delivery with intact membranes, 53 cases)

	Increasing or steady frequency	Decreasing frequency	
		n	Total %
Placebo	16	12	42.8
Utrogestan	3	22	88

oxytocin, prostaglandin, and  $\alpha$ -adrenergic stimulation while being devoid of any known toxicity.<sup>2,3,10</sup>

These potential advantages, however, have not yet been tested convincingly in true human therapeutic conditions. Some progesterone derivatives, such as 17  $\alpha$ -OH-progesterone or chlormadinone acetate, have been used; however, their binding to human myometrium progesterone receptors of microsomal membranes is very weak, and their efficacy could be quite different from that of the natural hormone.

The development of a galenic form permitting oral administration of natural progesterone enhances the interest of this treatment, since its administration is simple. This formulation with the micronized steroid has previously demonstrated its ability to increase both the plasma and myometrial levels of progesterone in late human pregnancy.<sup>11</sup>

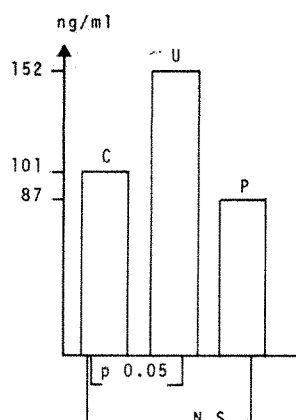
Our study permits the double-blind comparison of the tocolytic efficacy of a single oral dose of 400 mg of progesterone to that of a placebo.

The two groups of women who received at random either the placebo or oral progesterone did not differ significantly in age, obstetric antecedents, or severity of risk of premature delivery on admission.

In the hour following administration of a placebo, 42.8% of patients presented clinical improvement (Tables I and II). This percentage is identical to that in other studies concerning premature labor.<sup>10</sup>

Thus rest, associated or not with a placebo, has a tocolytic effect in almost half of the nonselected population attending an obstetric clinic for risk of premature delivery. This effect probably correlates with a decrease in adrenergic stimulations together with a decline in adrenal production of glucocorticoids and dehydroepiandrosterone: These two hormones, the production of which increases during stress, appear capable of inhibiting progesterone synthesis in the placenta and fetal membranes.<sup>12-15</sup> Rest and use of a placebo may help to increase progesterone concentration, at least locally in the myometrium and fetal membranes, whereas all circumstances of stress will cause the opposite effect.

In support of this hypothesis we noted that when administration of a placebo coincided with clinical im-



**Fig. 1.** Levels of plasma progesterone during treatment for risk of premature delivery. C, Control levels before treatment; P, 1 hour after administration of placebo; U, 1 hour after administration of Utrogestan (400 ml of oral progesterone); NS, not significant.

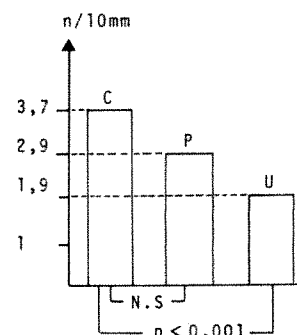
**Table III.** Levels of plasma progesterone in the presence or absence of clinical improvement in cases with risk of premature delivery

	With clinical improvement	Without clinical improvement
Cases with increasing progesterone levels (%)	70	35
Cases with steady or decreasing progesterone levels (%)	30	65

provement, plasma progesterone increased in 78% of cases, but it increased in only 17% when there was no clinical improvement.

In contrast to the use of a placebo, the oral administration of a single oral dose of 400 mg of progesterone initiated a significant increase in plasma progesterone of about 50% after 1 hour in all patients of the treated group (Fig. 1). This increase coincided in general with a decline in uterine contractions, which was significantly superior to that obtained with a placebo (Fig. 2) and corresponds to a sufficient therapeutic efficacy in 75% to 88% of cases, depending on the initial severity of the premature labor (Tables I and II). In nearly 30% of cases, however, there was no correlation between clinical improvement and levels of plasma progesterone (Table III). This suggests that the hormone variations of the plasma compartment are not the determining element of the tocolytic mechanism.<sup>16</sup> It is likely that the concentration relationship between estradiol and progesterone, in the myometrium and fetal membranes, would be a far more precise element for assessing the clinical improvement.<sup>13, 15-17</sup>

Furthermore, progesterone plasma levels of preg-



**Fig. 2.** Evolution of the frequency of uterine contractions 1 hour after administration of placebo or oral progesterone. C, Control levels before treatment; P, 1 hour after administration of placebo; U, 1 hour after administration of Utrogestan (400 ml of oral progesterone); NS, not significant.

nant women are unstable during a 24-hour period and a single daily blood sample is not sufficient to adequately evaluate the progesterone production of each individual woman.<sup>15b</sup>

In the majority of cases, oral progesterone provokes a decline in uterine activity which is less dramatic than that usually observed with use of intravenous  $\beta$ -mimetics. However, in the majority of cases this effect is strong enough to achieve the therapeutic effect required (that is, no premature delivery) without any side effect except for slight occasional drowsiness. In only three cases was the single oral dose of progesterone clearly insufficient when compared to intravenous  $\beta$ -mimetics, but it remains possible that an intravenous preparation of progesterone would produce the same intensity of effect as that of a  $\beta$ -mimetic. Finally, it should be noted that the mechanisms of action of progesterone and of  $\beta$ -mimetics do not counteract each other but are complementary. Progesterone inhibits the  $\alpha$ -adrenergic receptors without modifying the  $\beta$ -receptors, the clinical expression of which may thus become predominant.<sup>5</sup> Therefore, in the most severe cases of premature labor use of oral progesterone could probably lower the minimal active dose of  $\beta$ -mimetic, thus decreasing the side effects of the treatment. Further investigations are currently in progress to assess this latter therapeutic scheme.

In conclusion, with the current conditions of selection in obstetric clinics, approximately 10% of cases with risk of premature delivery are beyond all medical treatment and 40% have a spontaneous favorable response with rest, associated or not with a placebo. Therefore, this means that 50% of the cases require efficient medical therapy and, if possible, without side effects. Use of oral progesterone, at least in specific galenic formulation (Utrogestan), permits a sufficient beneficial effect in about nine of 10 of these cases. Even though the decline in uterine activity is obtained less rapidly

and less intensely than with use of intravenous  $\beta$ -mimetics, the safety of oral progesterone makes it the first choice for treatment of risk of premature delivery of average severity. For the most severe cases the association of oral progesterone and intravenous  $\beta$ -mimetics probably presents great therapeutic advantages. Finally, if the clinical improvement after progesterone or placebo administration generally coincides with an increase in levels of plasma progesterone, this is not true in each individual case. This confirms the fact that during the last 3 months of pregnancy, hormonal exploration of the vascular compartment does not deliver precise information about the local situation of the myometrium and fetal membranes.

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# Uterine activity during pregnancy in ambulatory patients: Comparison of singleton and twin gestations

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Prelabor uterine activity was monitored daily in a group of ambulatory outpatients who were delivered at term. The study included 22 patients with one fetus and 18 with twin gestations. The mean weekly frequency of uterine activity during twin gestations was found to be significantly higher throughout pregnancy than that identified during pregnancies with a single fetus. In twin gestations a gradual significant rise in frequency of contractions could be observed with advancing gestational age. The data presented could help establish the guidelines for evaluation of uterine activity records during pregnancies complicated by a multifetal gestation. (AM J OBSTET GYNECOL 1986;154:530-1.)

**Key words:** Uterine activity, single and twin gestation.

Numerous studies have shown that increased prelabor uterine activity precedes the development of preterm labor.<sup>1-3</sup> Although each of these reports established certain guidelines for the normal pattern of uterine activity during pregnancy, none of them noted whether differences existed between unifetal and multifetal gestations. Furthermore, these were studies of women who were either hospitalized or otherwise limited in their activities. Since most women ambulate during gestation and since multifetal gestations are known to have an increased incidence of preterm labor, it seems important to determine whether the normal baseline uterine activity in these pregnancies differs from that of gestations with a single fetus.

In the following study daily monitoring was performed at home in a group of ambulatory patients with unifetal and multifetal gestations, all of whom were delivered at term. The results indicate that multifetal gestations are associated with increased uterine activity throughout pregnancy even in the absence of preterm labor.

## Methods

Forty gravid women were monitored by means of a recently developed lightweight tocodynamometer designed for ambulatory outpatient use.<sup>4</sup> A detailed description of this monitor, the accuracy of its recordings, and mode of operation have been previously reported.<sup>4,5</sup> Uterine activity was monitored two to four

times daily for a cumulative total of at least 200 minutes per day, and the data were stored in the device's memory. These data were transmitted daily via the telephone to tocographs located in the study center.

Outpatient prenatal care was provided every 1 to 3 weeks and included a pelvic examination for evaluation of cervical status. Only subjects who were delivered at or beyond 36 completed gestational weeks were included. Eighteen of the patients had a twin gestation and 22 had a single fetus. Selected clinical characteristics are given in Table I.

In a review of daily transmissions, only contractions lasting >35 seconds were included in the analysis. Contractions of shorter duration, most of which were of the low-amplitude high-frequency type, were excluded from analysis. The mean weekly frequency of contractions was calculated from each patient's daily average frequency and plotted at the appropriate gestational age. Gestational age was determined from considering the last menstrual period, physical examination, ultrasonography, and neonatal evaluation. Comparisons of the mean weekly frequencies of uterine activity within the same group were done by analysis of variance for repeated measures. Comparisons of the mean weekly frequencies between the two groups at any particular gestational age were done with use of the unpaired *t* test. The results are expressed as means  $\pm$  SD.

## Results

The mean weekly frequency of contractions according to gestational age is given in Fig. 1. For the multifetal gestation group, each point in the figure represents at least 80 observations on at least 14 patients. For the singleton group, each point represents at least 100 observations on at least 18 patients. The differences between the two groups were significant throughout

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**Table I.** Clinical characteristics of study sample (mean  $\pm$  SD)

	Maternal age (yr)	Multi-parous	Primi-parous	Duration of monitoring (wk)
Twins (N = 18)	31.5 $\pm$ 4.5	7	11	12 $\pm$ 2.5
Single (N = 22)	29.5 $\pm$ 4.8	8	14	10.8 $\pm$ 2.7

the observation period. A progressive significant rise in frequency of contractions could be seen among the twin pregnancies. In patients with a single fetus a statistically significant rise in contraction frequency could only be seen in the thirty-sixth week of pregnancy.

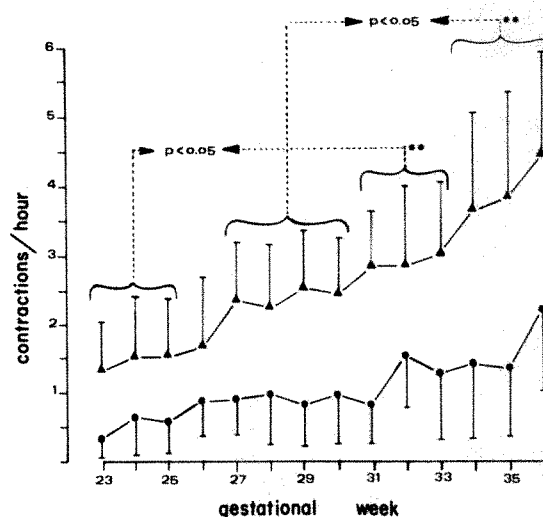
### Comment

Multifetal gestations are known to be associated with a higher incidence of preterm labor and preterm births. Previous studies that included both multifetal and single gestations have shown excessive uterine activity before the development of preterm labor.<sup>1,3</sup> Of note, however, is the fact that relatively little attention has been given to description of uterine activity associated with term deliveries in pregnancies with one or more fetuses. In a recent study of ambulatory patients who underwent tocodynamometry at home, a few patients were observed to have an increased frequency of contractions without premature labor (that is, progressive cervical dilation and effacement).<sup>6</sup> There was a clinical impression that among the patients with "false preterm labor," women with multifetal gestations have been overrepresented and often overtreated by uterine relaxants. Therefore the clinical importance of establishing the frequency of uterine activity during normal pregnancy in these high-risk patients is not only academic but may also be of clinical significance.

A progressive significant increase in frequency of contractions before 36 weeks' gestation was observed among multifetal but not single fetus pregnancies. Although this study did not include monitoring beyond 36 completed gestational weeks, Zahn<sup>7</sup> has recently reported a similar rise after 36 weeks' gestation in singleton pregnancies.

Of particular interest is the fact that the frequency of uterine activity which was observed by us in ambulatory patients was quite similar to that reported by others for patients who were resting.<sup>1-3</sup> Although this may indicate that bed rest has little effect on uterine activity in normal patients, the effect of bed rest in patients with preterm labor needs further study.

The etiology of the differences in uterine activity



**Fig. 1.** Frequency of contractions (mean  $\pm$  SD) in ambulatory patients with singleton (circle) and twin (triangle) gestations. \* -  $p < 0.05$  as compared to all preceding weeks. \*\* -  $p < 0.05$  as compared to the weeks indicated by brackets.

between single and multifetal gestations remains speculative but includes such possibilities as altered uterine tone secondary to overdistension, altered hormonal milieu resulting from the imbalance of the fetal-placental ratio, or increased fetal activity produced by two fetuses. Whatever the source of this difference may be, its existence must be noted as a potential source of confusion for those interpreting patterns of prelabor uterine activity. It is likely that threshold values must be reestablished to define the difference between physiologic and pathologic frequencies of uterine activity for women with twin gestations. In this way the real risk of preterm labor and birth can be better assessed for the patient with a multifetal gestation.

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# Antenatal diagnosis of renal anomalies with ultrasound

## IV. Bilateral multicystic kidney disease

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Bilateral multicystic kidney disease is a congenital disorder that is fatal in the newborn period. A series of nine cases of bilateral multicystic kidney disease diagnosed prenatally by ultrasound is presented. Ultrasound criteria necessary for the diagnosis are (1) bilateral multicystic kidneys, (2) loss of renal architecture, (3) nonvisualization of the fetal bladder, and (4) absence of amniotic fluid. Seven of the nine cases had autopsy confirmation of the diagnosis. Three cases had other associated congenital anomalies. Precise prenatal diagnosis may allow patients the option of elective abortion or may prevent unnecessary obstetric intervention. We propose that a reliable diagnosis can be made with prenatal ultrasound. (AM J OBSTET GYNECOL 1986;154:532-7.)

**Key words:** Bilateral multicystic kidney disease, ultrasound diagnosis, congenital anomalies

Bilateral multicystic kidney disease is a disorder that is fatal in the newborn period.<sup>1</sup> Multicystic kidney disease has been called polycystic kidney disease, multicystic dysplastic kidney disease, and dysplastic kidney disease. In this communication multicystic kidney disease refers to the type II form of Osathanondh and Potter's classification<sup>2</sup> of polycystic disease of the kidney. Type II multicystic kidney disease is usually bilateral.<sup>1</sup> Less often only one kidney or a segment of one kidney is affected. The kidneys may be normal or increased in size (type IIA) or reduced in size (IIB). On gross examination the affected kidneys may be likened to a "cluster of grapes" with complete loss of normal reniform shape. Histologically, thick-walled cysts lined by cuboidal or columnar epithelium, surrounded by immature mesenchymal stroma, are seen. Islands of cartilage, abnormal glomeruli, and fibroblastic proliferation may also be identified.

Since bilateral multicystic kidney disease is fatal in the newborn period,<sup>1</sup> the importance of precise prenatal diagnosis of this condition is twofold. First, prenatal diagnosis is possible early enough in gestation to allow patients the option of elective abortion. Second, if the diagnosis is made in the late second trimester or the third trimester, it may prevent unnecessary obstetric intervention. In an effort to test the hypothesis that

a reliable prenatal diagnosis can be made with ultrasound, this report reviews our experience during a 4-year period in the diagnosis of this rare but lethal disease.

### Material and methods

For the time period January 1, 1980, to April 1, 1984, the following records were reviewed: (1) ultrasound records in which the primary diagnosis was multicystic kidney disease; (2) ultrasound records in which other congenital anomalies were the primary diagnosis, to determine if multicystic kidney disease was also diagnosed; (3) autopsy records of perinatal deaths, to confirm the prenatal ultrasound diagnosis of multicystic kidney disease and to determine if prenatal sonography had missed or incorrectly diagnosed multicystic kidney disease.

Sonographic examinations were performed with a static B scanner with a 3.5 MHz transducer (Picker, North Haven, Connecticut) and/or linear array real-time equipment with a 3.5 MHz transducer (General Electric, Milwaukee, Wisconsin; ADR, Tempe, Arizona; Picker; Toshiba SAL SOA, Tustin, California) or a mechanical sector scanner (ADR).

Our prenatal diagnosis was based on the ultrasound findings of (1) bilateral multicystic abdominal masses, (2) loss of normal renal architecture, (3) failure to visualize the fetal bladder, and (4) absence of amniotic fluid (Fig. 1).

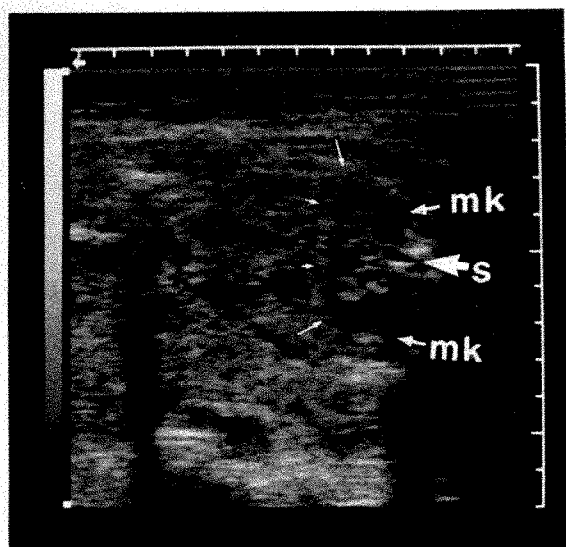
### Results

During this period nine cases of bilateral multicystic kidney disease and three cases of unilateral multicystic

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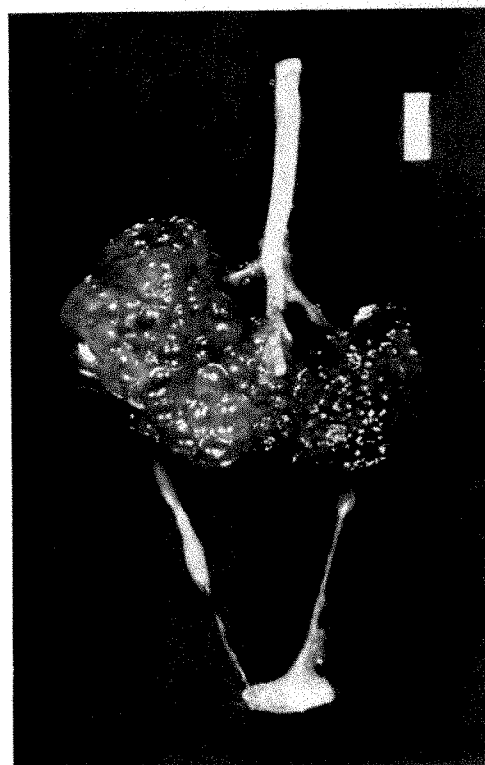
**Fig. 1.** Transverse scan through fetal abdomen. The multicystic kidneys (MK) are seen as bilateral masses filled with echo-free areas on both sides of the spine (S). Note the absence of amniotic fluid.

kidney disease were diagnosed prenatally. Pathologic confirmation was not available in the unilateral cases since operation was not performed to remove the diseased kidney. With one exception (Case No. 10), we have restricted our communication to bilateral multicystic kidney disease. Table I lists the pertinent clinical features for each case of bilateral multicystic kidney disease. Seven of the nine cases were diagnosed before 24 weeks. All seven patients elected to terminate the pregnancy. In the two cases diagnosed after 24 weeks, vaginal delivery occurred at 38 and 41 weeks, respectively.

Autopsy information was available in seven of the nine cases, and in all seven the prenatal diagnosis was confirmed. The gross and microscopic features from the autopsy of one of these infants are displayed in Figs. 2, 3, and 4, and these serve as classic examples of the pathologic characteristics of multicystic kidney disease.

Associated congenital anomalies were noted ultrasonically in three patients. In Case No. 3, the fetal stomach was not visualized and a diagnosis of tracheoesophageal fistula was entertained. In Case No. 5 there was evidence of ascites, pericardial effusion, and right ventricular hypertrophy. In Case No. 7 dilated lateral ventricles were observed. Autopsy confirmed the findings in Cases No. 3 and No. 5, but permission for autopsy was not given in Case No. 7.

A tenth case deserves special mention. In this patient ultrasound examination at 35 weeks of gestation demonstrated evidence of bilateral multicystic abdominal masses, loss of normal renal architecture, and nonvi-



**Fig. 2.** Anterior view of horseshoe-shaped multicystic kidneys in a 22-week fetus. Note the "cluster of grape" morphologic features and the concomitant bilateral ureteral abnormality. The proximal segments of the ureters are stenotic while the distal portions are atretic. In addition, the bladder is hypoplastic.

sualization of the fetal bladder. There was only a moderate degree of oligohydramnios. It was felt that a definitive diagnosis of bilateral multicystic kidney disease could not be made in the presence of amniotic fluid. Two differential diagnoses were considered: bilateral high-level ureteropelvic junction obstruction or a combination of multicystic kidney disease on one side and a ureteropelvic junction obstruction on the other. After delivery renal function studies were normal. An intravenous pyelogram demonstrated a high ureteropelvic junction obstruction on the left side and a nonfunctioning right kidney. A left ureteropyeloplasty was performed. The right kidney was thought to be multicystic and was not removed at the time of operation. A follow-up intravenous pyelogram at 3 years demonstrated a normally functioning left kidney and no visualization of the right kidney.

#### Comment

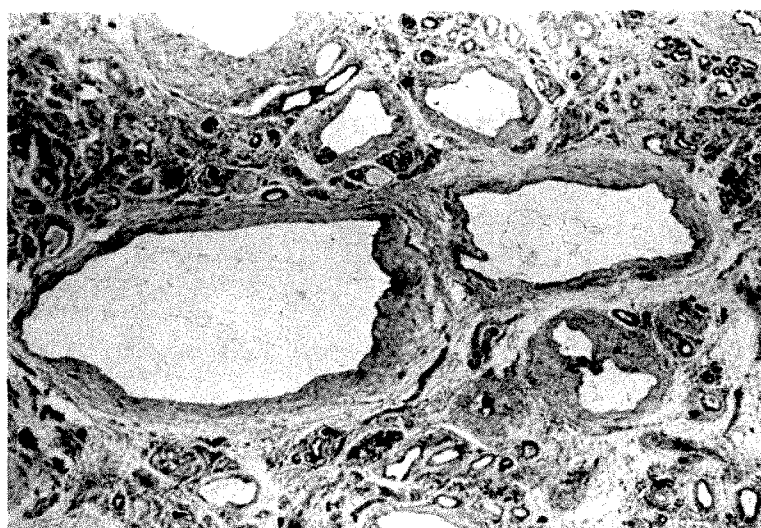
The prenatal diagnosis of many renal abnormalities is now possible. Obstructive uropathy,<sup>3</sup> renal agenesis,<sup>4</sup> and infantile polycystic kidney disease<sup>5</sup> have been the subject of previous reports from this unit. Currently in the literature are 10 case reports of unilateral multi-



**Table I.** Clinical and sonographic data in affected fetuses with bilateral multicystic kidney disease

Case No.	Age	Parity	Gestational age at diagnosis (wk)	Reason for scan	Ultrasound findings		
					Appearance of kidneys	Bladder	Amniotic fluid
1	18	Primiparous	18	Oligohydramnios, level I scan	Bilateral enlargement; multiple small cysts; largest cyst 3 × 1.8 cm	Absent	Not detectable
2	32	G <sub>2</sub> SAB <sub>1</sub>	17	Oligohydramnios, level I scan	Large cyst, 3.7 × 3.3 cm (L); large kidney with multiple small cysts (R)	Absent	Not detectable
3	28	G <sub>2</sub> SAB <sub>1</sub>	20	Oligohydramnios, level I scan	Right kidney, 4.0 × 2.7 cm with multiple cysts; left kidney, 1.0 × 1.0 with cysts	Absent	Not detectable
4	21	G <sub>1</sub>	21	Cystic mass, level I scan	Right kidney, normal size with cysts; large cystic structure (L), 7.5 × 8 cm	Absent	Not detectable
5	24	G <sub>2</sub> SAB <sub>1</sub>	23	Cystic mass, level I scan	Bilateral large kidneys replete with small cysts	Absent	Not detectable
6	28	G <sub>3</sub> P <sub>2</sub>	22	Previous infant with rhabdomyosarcoma	Bilateral large kidneys of equal size, replete with cysts	Absent	Not detectable
7	21	G <sub>2</sub> P <sub>1</sub>	18	Class F diabetes	Right kidney, enlarged with multiple cysts; left kidney, normal size with multiple cysts	Absent	Not detectable
8	31	G <sub>1</sub>	26	Oligohydramnios, level I scan	Bilateral enlargement of kidneys of equal size with multiple cysts	Absent	Not detectable
9	22	G <sub>1</sub>	35	Oligohydramnios, level I scan	Bilateral enlargement of kidneys of equal size	Absent	Not detectable

G = Gravidity; SAB = spontaneous abortions; P = parity; L = left; R = right.

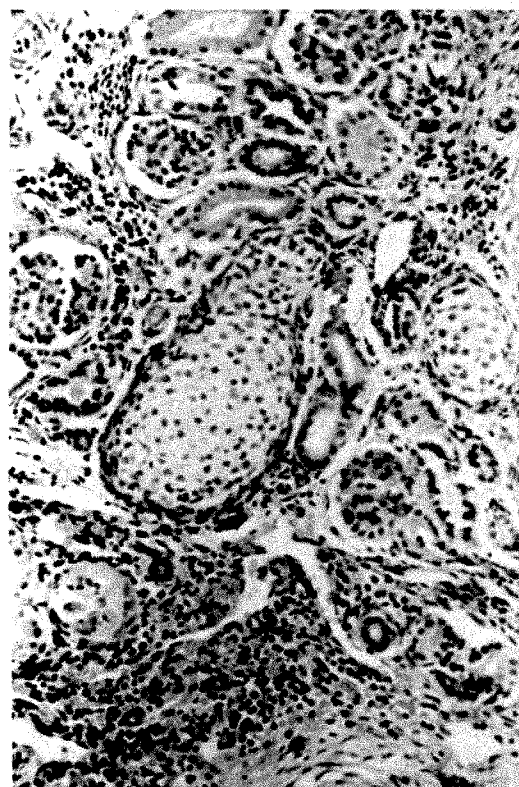


**Fig. 3.** Frozen section of Potter's type II or multicystic kidney. Note the thick-walled cysts with a rather flattened epithelium. In addition, immature mesenchymal stroma and tubules are seen.

<i>Pregnancy outcome</i>	
<i>Outcome</i>	<i>Autopsy findings</i>
Prostaglandin termination	Type II Potter's syndrome; combined kidney weight 18 gm
Prostaglandin termination	Type II Potter's syndrome; combined kidney weight 22 gm; 46,XY karyotype
Prostaglandin termination	Type II Potter's syndrome; tracheoesophageal fistula; congenital absence of radius and thumb bilaterally; 46,XY karyotype
Prostaglandin termination	Type II Potter's syndrome
Prostaglandin termination	Type II Potter's syndrome; hypoplastic left ventricle; hypertrophy of right ventricle; 46,XX karyotype
Prostaglandin termination	Type II Potter's 46,XY karyotype
Prostaglandin termination	Refused autopsy
Vaginal delivery 41 wk; infant died within 1 hr of birth; Potter's phenotype	Refused autopsy
Delivery at 38 wk; infant died within 1 hr of birth; Potter's phenotype	Type II Potter's syndrome; kidneys 29.5 gm; 46,XY karyotype

cystic kidney disease<sup>6-14</sup> and one case report of bilateral multicystic kidney disease.<sup>15</sup> This report deals with nine cases of bilateral multicystic kidney disease.

When the diagnosis of bilateral multicystic kidney disease is entertained, one must also consider infantile polycystic kidney disease and bilateral ureteropelvic junction obstruction. Both bilateral multicystic kidney disease and infantile polycystic kidney disease are associated with loss of normal renal architecture, absence of amniotic fluid, and absence of the fetal bladder on ultrasound examination. The kidneys in infantile polycystic kidney disease are large, homogeneous, hyperechogenic, solid masses (Fig. 5), whereas in multicystic kidney disease numerous echo-free areas are seen throughout the kidneys (Fig. 1). In infantile polycystic kidney disease the kidney circumference to abdominal circumference ratio is usually greater than 2 SD above the mean. In contrast, multicystic kidneys may be mildly or greatly enlarged (IIA) or even reduced in size (IIB) (Figs. 1 and 6). It should be noted, however, that in



**Fig. 4.** The presence of cartilage and interstitial lymphocytic infiltrates are additional features that may be seen in multicystic kidneys.

the early stages of infantile polycystic kidney disease the kidney size may be normal and amniotic fluid may be present.<sup>5</sup>

Hydronephrosis due to ureteropelvic junction obstruction can usually be clearly differentiated from multicystic kidney disease.<sup>17</sup> Particular attention must be paid to the renal architecture and identification of the renal pelvis. We found the following criteria useful for differentiation: (1) Several cystic masses separated by interfaces are seen in multicystic kidney disease whereas the dilated calyces in hydronephrosis are seen as hypoechogenic nonspherical areas radiating from the renal pelvis; (2) the largest cyst in multicystic kidney disease has a noncentral location; (3) in multicystic kidney disease one often fails to identify a definite renal parenchymal rim. Severe forms of hydronephrosis, however, may produce large cystic structures where no normal renal architecture is identified (Case No. 10) and may be extremely difficult to differentiate from multicystic kidney disease.

As with any form of prenatal diagnosis, the importance of accuracy in diagnosis cannot be overstated. Seven of the nine cases had autopsy-confirmed bilateral multicystic kidney disease. The remaining two cases in

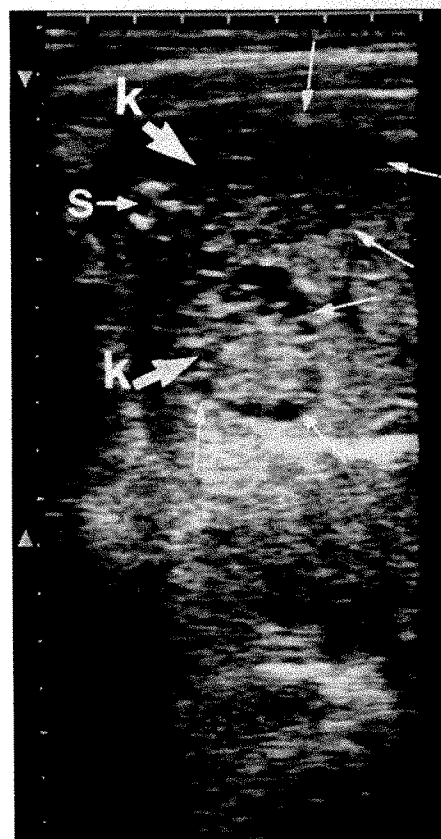


**Fig. 5.** Transverse scan demonstrating spine (S) and large polycystic kidneys (K). Note the loss of normal kidney architecture and the hyperechogenicity of the kidneys.

which autopsy was refused had the classic Potter phenotype, strongly suggesting that the diagnosis was correct. Additional reviews of the autopsy records in perinatal deaths did not identify any patient that had a missed diagnosis or incorrect diagnosis of multicystic kidney disease in the ultrasound unit. Because of the common association with other congenital anomalies, a more comprehensive scan is indicated. Detection may sometimes be difficult because of the absence of amniotic fluid. For example, in Case No. 3 the bilateral absence of the radius and thumb was not diagnosed prenatally.

The underlying pathogenesis of multicystic kidney disease appears to be a severe insult to the developing kidney before 8 to 10 weeks of gestation. The classic teaching is that this is a sporadic event with a very low recurrence risk.<sup>2</sup> However, Roodhooft et al.<sup>18</sup> have recommended that parents of children with bilateral renal agenesis and multicystic kidney disease be counseled about two risks, an increased risk (4.4% in their study) of another severely affected child in a subsequent pregnancy and a risk to themselves and siblings of having silent genitourinary malformations. It seems prudent to counsel patients about the uncertain risk of recurrence and the availability of prenatal diagnosis with ultrasound in the early second trimester for a future pregnancy.

The most striking feature that we noted from our experience was the total absence of demonstrable amniotic fluid in all nine cases of bilateral multicystic kid-



**Fig. 6.** This is another example of multicystic kidneys. There is a difference in size between the two kidneys and, when compared with those in Fig. 1, it is clear they are much larger in size.

ney disease. The tenth case described in this communication is one where the differential diagnosis from ureteropelvic junction obstruction was difficult. In retrospect, the most helpful ultrasonic finding was the presence of amniotic fluid. The presence of amniotic fluid indicates some kidney function and makes the diagnosis of bilateral multicystic kidney disease unlikely. In conclusion, an accurate prenatal diagnosis of multicystic kidney disease can be made with ultrasound.

We wish to thank Ingeborg H. Venus, M.S., for assisting in the collation of the above data.

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## Ultrasound differentiation of the competent from the incompetent cervix: Prevention of preterm delivery

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To evaluate the feasibility of the use of serial ultrasound measurements of cervical length, membrane protrusion, and dilatation to discriminate between the competent and the incompetent cervix, 107 at-risk patients and 30 control subjects were examined prospectively. Patients were divided into five groups based on treatment and method of diagnosis. Epidemiologic, ultrasound, and outcome data were analyzed. Means and standard deviations for ultrasound measurements were established. Highly significant differences between all prediagnostic and postdiagnostic-pretreatment measurements were found ( $p < 0.001$ ). Highly significant differences were also found between all postdiagnostic-pretreatment and postdiagnostic-posttreatment measurements ( $p < 0.001$ ). No significant differences between prediagnostic and postdiagnostic-posttreatment measurements were noted. The incidence of preterm delivery was significantly higher among untreated diagnosed patients ( $p < 0.01$ ). By combined clinical and ultrasound criteria 51 patients (47.7%) were identified as not having cervical incompetency. Fifty-six patients (52.3%) were diagnosed. (*AM J OBSTET GYNECOL* 1986;154:537-46.)

**Key words:** Incompetent cervix, sonographic measurement, preterm birth

The diagnosis of cervical incompetency is routinely accomplished by reviewing the clinical history of multigravid patients at risk.<sup>1</sup> If a classic history and/or previous cerclage is not noted, the clinician must rely on

waiting for either the appearance of premonitory symptoms or changes on pelvic examination. At best these methods of diagnosis are often unreliable and subjective, and they are frequently observed late in the developmental time course of this condition. The need to rely on past history to determine whether patients are at risk places the primigravid patient with congenital incompetency in a more vulnerable position. Quite often the diagnosis is missed entirely.

Anatomically, about half of the cervix is not accessible by routine vaginal examination. It is therefore not unreasonable to expect that anatomically short cervixes

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**Table I.** Frequency of historical risks

Rank	Historical risks	n	%
1	Spontaneous second-trimester loss	22	20.6
2	Preterm labor	20	18.7
3	Induced abortion	18	16.8
4	Suspicious or poor history	18	16.8
5	Premature rupture of the membranes	17	15.9
6	Twins	15	14.0
7	Previous dilatation	13	12.1
8	Miscellaneous	11	10.3
9	History of diethylstilbestrol exposure	10	9.3
10	Previous cerclage	7	6.5
11	Classic history	7	6.5
12	Habitual loss	6	5.6
13	Short cervix	6	5.6
14	Cervical trauma	6	5.6
15	Cold cone	6	5.6
16	Labor abnormalities	5	4.7
17	Myoma	5	4.7
Total	Historical risks	192*	179.4

\*Most patients had more than one risk. All patients had at least one risk.

when diagnosed by vaginal examination would be competent, whereas anatomically appropriate cervixes by vaginal examination may be incompetent but undiagnosed because significant structural changes remain hidden from the examiner's view above the vaginal vault. At the present time many physicians, rather than miss the diagnosis, will recommend prophylactic cerclage placement for patients at risk without classic histories or appropriate anatomic changes. Unfortunately, such a policy leads to overutilization of a procedure that is not without risk.<sup>2</sup> Ultrasound anatomy of the cervix has been defined to some extent, and it has been documented that ultrasound examination of the cervix can demonstrate the most advanced changes of cervical incompetency.<sup>3</sup>

To our knowledge, however, there has been no systematic ultrasound study of cervical anatomy in pregnancy designed to prospectively discriminate between the competent and incompetent cervix. The purpose of this article is to report a work-in-progress designed to demonstrate the clinical and technologic feasibility of use of ultrasound-documentable cervical changes that could diagnose the development of cervical incompetency regardless of past history or number of previous pregnancies. It was anticipated that by recognition of sonographic changes at the level of the internal os, the earliest possible changes of cervical incompetency could be documented and used clinically for patient management. Alternatively, by documenting normal cervical anatomy sonographically, the diagnosis of cervical incompetency could be excluded and unnecessary cerclage placement avoided. Furthermore, it was anticipated that significant differences in sonographic measurements before diagnosis, after diagnosis but be-

fore treatment, and after treatment could be documented.

### Material and methods

A total of 107 multigravid and primigravid patients at risk for cervical incompetency were selected from the high-risk practice of one of us (W. H. M.) or from the high-risk clinic supervised by him at Providence Hospital, Southfield, Michigan, to undergo serial ultrasound and pelvic examinations. Seventeen risk categories were identified and were, in declining order of frequency, spontaneous second-trimester losses, previous preterm labor, suspicious or poor pregnancy history, previously induced abortions in the first or second trimester, premature rupture of the membranes, multiple pregnancies, previous surgical dilatation, miscellaneous risks, and exposure to diethylstilbestrol with/ or without partial vaginectomy (Table I).

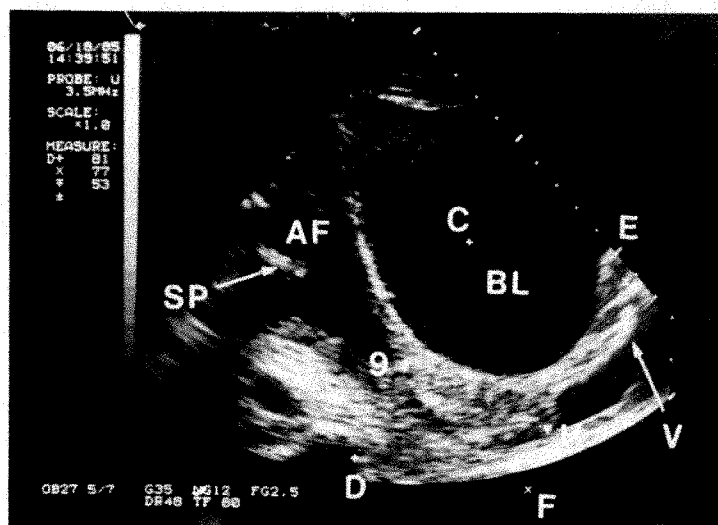
Patients included in this report were examined prospectively and surgically treated if necessary between January 1, 1983, and June 30, 1985. A control group of 30 low-risk multigravid and primigravid patients were concurrently followed. Serial ultrasound examinations in this group were indicated for reasons other than cervical incompetency.

At-risk patients were examined through a total of 480 ultrasound studies. These studies were usually performed at 7-day intervals. A few patients, however, were examined at 14- or 21-day intervals. Most patients were examined at least four times. The control patients were examined sonographically 120 times on at least three occasions between 14 to 28 weeks. Ultrasound examinations were done by one of us (W. H. M., J. K., or J. T.). Each patient admitted to the study gave appropriate informed consent. No patient was denied cerclage placement solely on the absence of ultrasound criteria. Patients found to be candidates for surgical intervention had a final ultrasound examination within 24 hours of treatment or in the operating room before cerclage.

Patients with negative ultrasound examinations, non-confirmatory symptoms, and/or vaginal changes were not subjected to cerclage. All patients who developed significant or classic changes on pelvic examination, regardless of ultrasound findings, were treated surgically.

For the purposes of comparison at each examination, multiple measurements were taken from the cervical sonograms, and the average of a particular measurement was reported. Membrane protrusion was divided into two subsets for descriptive purposes. Cervical nipping represented membrane protrusion through the internal os for an arbitrarily defined distance of  $\leq 6$  mm. Membrane herniation represented protrusion of the membranes through the internal os for a distance of  $>6$  mm.

To measure the cervical length, a line was drawn



**Fig. 1.** Base of the internal os. AF = Amniotic fluid, V = (arrow) vagina with water, H = external os, g-H = cervical length, BL = bladder, G = internal os, CgD = plane of decidual plate, EHF = plane of external os—pars vaginalis, and SP = small parts.

through the center of the cervical canal, which is often represented sonographically by an anechoic space in the center of the cervix, from the vaginal side of the decidual plate or the base of the membranes in front of the sonographic internal os to the base of the sonographic external os. If the decidual plate was present, the length of the cervix was measured only to the vaginal side of the decidual tissue that separates the membranes from the internal os. The base of the internal os was identified as the apex of the membrane U-formation covering the cervix (Fig. 1).

Initially it was hoped to divide the population into a treatment and nontreatment group based on ultrasound-observable changes. Early in the study we realized that ultrasound-observable changes of cervical incompetence, when left untreated, could result in second-trimester losses. The study design, therefore, could not incorporate a randomized treatment and nontreatment group.

The total sample of 137 patients was divided into the following five groups based on treatment and method of diagnosis: Group 1 consisted of treated patients who were diagnosed because of a classic history, previous cerclage, or obvious hourglassing of the membranes. Patients in this group did not always have early sonographic changes of cervical incompetence. Group 2 consisted of treated patients who were found to have sonographically observable changes of cervical incompetence. In this group the clinical symptoms or the results of serial vaginal examinations were inconclusive. Group 3 consisted of those patients who were diagnosed both by ultrasound and/or clinical criteria to be incompetent but could not be treated. Group 4 consisted of patients who were judged to be competent by ultrasound criteria in spite of worrisome clinical his-

tories and/or inconclusive changes on pelvic examinations. Group 5 consisted of the control patients, who did not demonstrate sonographic changes of incompetence, significant historical risks, or changes on pelvic examination. Patients in Groups 1 through 4 were at high risk for developing cervical incompetence. Group 5 patients were at low risk (Table II).

**Statistical analysis of data.** The five groups previously defined were compared according to the number of risk factors, age, gravidity, parity, and past history of premature births to determine whether or not there were intergroup differences that might have affected the ultrasound data. These groups were also compared by the outcome measures of birth weight, gestational age at birth, and 1- and 5-minute Apgar scores.

The intent of the analysis was to demonstrate that an at-risk population could be screened for cervical incompetence by ultrasound criteria. Group differences were evaluated by analysis of variance and  $\chi^2$  analysis. Changes in ultrasound measurements over time were analyzed by repeated measures analysis of variance.

## Results

The 107 patients who were at risk of developing cervical incompetence were examined for frequency of risk factors. As seen in Table I, most patients had more than one risk factor.

The mean age of the sample was 28.8 years (range, 15 to 40 years). Of the 107 at-risk patients, 90 were multigravida and 61 had one or more first- or second-trimester losses. Among these patients, 11 had three or more losses. One or more first-trimester voluntary terminations of pregnancy were reported by 18 patients (Table III).

**Table II.** Analysis of outcomes according to diagnostic groups

Diagnostic group	Description	n	%	At risk	Cervical incompetency	Positive ultrasound findings	Positive history	Positive symptoms	Positive examination
1	Treated; positive clinical diagnosis; positive history	14	13.1	+	+	+/-	+	+	+
2	Treated; positive ultrasound-assisted diagnosis	32	29.9	+	+	+	+/-	+/-	+/-
3	Untreated; inconclusive ultrasound-assisted diagnosis	10	9.3	+	+	+/-	+/-	+	+
4	Untreated; negative ultrasound-assisted diagnosis	51	47.7	+	-	-	+/-	+/-	+/-
Subtotal		107	100	+	56	32			
5	Untreated; control subjects; ultrasound-assisted diagnosis	30		-	-	-	-	-	-
Total		137							

+ = Yes. - = No. +/- = Inconclusive.

**Table III.** Selected historical and outcome factors by analysis of variance\*

Category	Group 1 (n = 14)*	Group 2 (n = 32)	Group 3 (n = 10)	Group 4 (n = 51)	Group 5 (n = 30)	Probability
Age (yr)	29.6 ± 5.6	28.3 ± 4.6	26.9 ± 7.2	29.2 ± 4.6	30.8 ± 5.2	NS†
Gravidity	3.6 ± 1.8	3.9 ± 1.8	3.3 ± 4.6	3.2 ± 2.2	2.5 ± 1.0	NS
Parity	1.4 ± 1.1	1.2 ± 1.2	1.1 ± 1.9	0.9 ± 1.1	0.8 ± 0.7	NS
First- or second-trimester abortions	1.3 ± 1.3	1.3 ± 1.3	1.2 ± 2.8	1.0 ± 1.3	0.6 ± 0.8	NS
History of prematurity	0.8 ± 1.0	0.7 ± 1.0	0.5 ± 0.7	0.1 ± 0.3	0.03 ± 0.2	0.001
Birth weight (gm)	2532 ± 1493	2740 ± 776	1705 ± 1268	3028 ± 883	3473 ± 634	0.001
1 min Apgar score	5.8 ± 3.7	7.2 ± 1.4	5.5 ± 3.9	7.8 ± 1.8	8.1 ± 0.8	0.005
5 min Apgar score	7.0 ± 3.5	8.5 ± 0.8	6.0 ± 4.2	8.3 ± 2.0	8.9 ± 4.4	0.001
Gestational age at delivery (wk)	34.7 ± 6.7	36.3 ± 4.0	30.1 ± 8.1	36.1 ± 5.7	38.7 ± 1.5	0.001
Risk factors	2.0 ± 1.0	2.0 ± 0.8	1.6 ± 1.0	1.7 ± 0.8	—	NS

\*Contrasts of the individual groups were done by a modified Newman-Keuls procedure.

†Results are given as mean plus or minus standard deviation.

‡NS = Not significant.

Symptoms of cervical incompetency were documented in 33 of the 107 at-risk patients (Table IV). Pelvic findings were documented in 58 patients (Table V). Hourglassing of the membranes developed in eight (7.5%) of the patients. Of the 56 patients diagnosed as having cervical incompetency, eight (14.3%) developed hourglassing. Of these eight, six developed advanced membrane protrusion before being scheduled for surgery and the other two developed hourglassing after being diagnosed and scheduled for surgery. Hourglassing was noted during the first clinical examination in four of the eight patients.

Premature rupture of the membranes was most common among group 3 patients (50%), less common among group 1 and 2 patients (11.1%), and least common among group 4 patients (3.9%). The mean gestational age of diagnosis of cervical incompetency was  $20.6 \pm 4.9$  weeks (range, 14 to 30 weeks). Of the study population, 40 (43%) of the patients were diagnosed between the sixteenth and twenty-sixth week of preg-

nancy inclusively. By the twenty-eighth week of gestation, 45 (92.2%) of all possible diagnoses of cervical incompetency had been made.

Of the 107 patients at risk, 56 (52.3%) were diagnosed as having cervical incompetency (groups 1, 2, and 3). Diagnosis of cervical incompetency was excluded in the other 51 (47.7%) (group 4). Of the diagnosed patients, 14 (25%) were diagnosed primarily by clinical means (group 1), 32 (57.1%) were diagnosed primarily by ultrasound findings (group 2), and only the 10 untreated patients (17.9%) were diagnosed variably by ultrasound, clinical symptoms, and examination (group 3). Of the 30 control subjects (group 5), there was no documentable evidence of cervical incompetency, either by ultrasound or clinical examination (Table II). Of the 56 diagnosed patients, 46 (82.1%) were treated with either a modified Shirodkar procedure (31, or 67.3%) or a McDonald's suture (14, or 30.4%) or given hydroxyprogesterone caproate (one, or 2.2%).

<i>Treated</i>	<i>Delivered</i>		<i>Undelivered</i>		<i>Term (n)</i>	<i>Premature (n)</i>	<i>VLBW (n)</i>	<i>Second-trimester abortion at 20 wk</i>	<i>Treatment</i>
	<i>n</i>	<i>%</i>	<i>n</i>	<i>%</i>					
+	10	71.4	2	14.3	7	3	1	2	+
+	25	83.1	7	21.9	20	5	2	0	+
-	6	60.0	0	0	2	4	1	4	-
-	44	91.2	6	11.8	37	7	3	1	-
46	85	79.4	15	14.0	66	19	7	7	
-	30	100	0	0	26	4	0	0	-

**Table IV.** Frequency of symptoms

<i>Rank</i>	<i>Symptoms</i>	<i>n</i>	<i>%</i>
1	Increased pressure	24	22.4
2	Increased discharge	10	9.3
3	Bloody discharge	7	6.5
4	Dysuria	2	1.9
5	Bleeding	2	1.9
6	Cramps	1	0.9
Total		46*	

Some patients had more than one symptom; 74 of 107 had no symptoms.

Since this article describes a work-in-progress as of June 30, 1985, 92 of the 107 patients (86%) either have been delivered or aborted. The premature delivery rate, abortion rate, and term delivery rate of groups 1 through 5 are listed in Table II.

The perinatal mortality rate for the 85 delivered patients in this study was 35.3 per 1000 live births (uncorrected). Of the three cases of perinatal mortality, one neonate, delivered from a group 2 patient, died because of  $\beta$ -streptococcal pneumonia. Another very low birth weight infant was delivered from a group 4 patient and died because of extreme prematurity. Preterm labor in this case was felt to be secondary to complications of placenta previa and degenerating myoma. A third neonate delivered from a group 3 patient who was diagnosed correctly could not be treated because of premature rupture of the membranes. These losses would not have been prevented by early diagnosis of cervical incompetence. The incidence of very low birth weight deliveries among diagnostic groups 1 through 4, respectively, was 10.0%, 8.0%, 16.6%, and 6.8%. There were no very low birth weight deliveries in the control sample (Table II).

When the five diagnostic groups were compared on the specific epidemiologic variables of age, gravidity,

**Table V.** Frequency of pelvic findings

<i>Rank</i>	<i>Pelvic findings</i>	<i>n</i>	<i>%</i>
1	Short cervix	36	33.6
2	Patulous cervix	20	18.7
3	Diethylstilbestrol exposure changes	5	4.7
4	Deformed cervix	5	4.7
5	Dilatation	2	1.9
6	Bulging lower uterine segment—cervical complex	2	1.9
Total		70*	

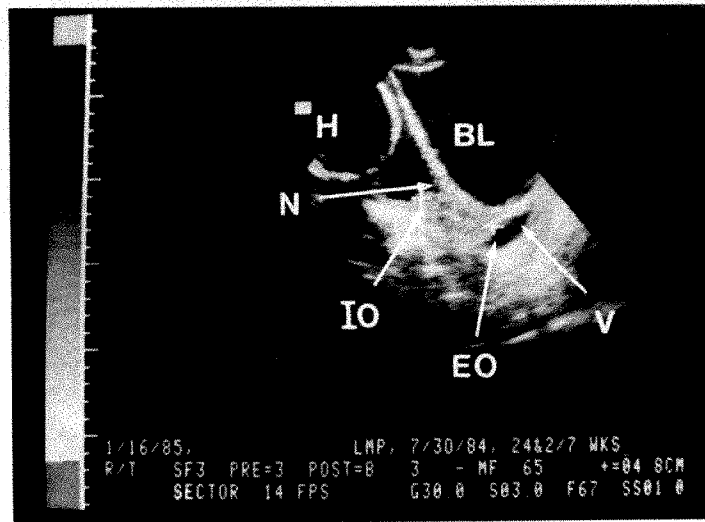
\*Some patients had more than one pelvic finding.

number of risk factors, and history of previous spontaneous abortions in the first and second trimester, no significant differences between the variables were found except for past history of premature births. Not surprisingly, groups 1 through 4 had significantly higher rates of previous premature births than the control group ( $p < 0.001$ ). In addition, groups 1 and 2 had a significantly greater incidence of previous preterm deliveries than groups 3 and 4 ( $p < 0.001$ ).

As seen in Table III, the five diagnostic groups differed significantly ( $p < 0.001$ ) in the following outcome measures: gestational age at delivery, birth weight, and 1- and 5-minute Apgar scores. At-risk groups 1, 2, 3, and 4 gave birth to a significantly larger proportion of low birth weight infants compared to the control group. In addition, group 3 had a significantly greater incidence of low birth weight infants when contrasted to group 4. Similarly, the control group had significantly greater mean 1- and 5-minute Apgar scores as well as infants of older gestational ages with larger mean birth weights when contrasted to the at-risk patients (groups 1 through 4). Also, infants of group 4 patients had significantly higher mean birth weights than those of group 3 (Table III).

**Analysis of ultrasound data.** To evaluate the dis-





**Fig. 2.** Normal cervix with cervical nipple. *H* = Fetal head, *IO* = sonographic internal os, *V* = (arrow) vagina with water, *N* = nipple, *EO* = sonographic external os, and *BL* = bladder.

**Table VI.** Ultrasound-measurable cervical changes by analysis of variance—groups 1, 2, and 3

Group	n	Cervical length (mm)		Membrane protrusion (mm)*		Cervical dilatation (mm)		Probability†
		Prediagnosis	Postdiagnosis-pretreatment	Prediagnosis	Postdiagnosis-pretreatment	Prediagnosis	Postdiagnosis-pretreatment	
1	5	42.4 ± 8.0	26.8 ± 13.7	0.0 ± 0.0	29.0 ± 24.6	0.0 ± 0.0	18.0 ± 13.5	0.001
2	18	44.8 ± 10.0	32.0 ± 11.5	2.7 ± 3.6	17.1 ± 13.3	3.1 ± 6.3	16.9 ± 10.0	0.001
3	4	44.4 ± 8.0	30.0 ± 11.1	6.0 ± 2.7	23.8 ± 13.6	3.7 ± 7.1	16.4 ± 8.2	0.001
Total	27	44.3 ± 9.2	30.3 ± 11.1	2.7 ± 4.5	20.3 ± 15.9	2.6 ± 5.8	17.1 ± 10.1	0.001

\*By definition, cervical nipple equals membrane protrusion of ≤6 mm and membrane herniation equals membrane protrusion of >6 mm.

†Probability statements are for the differences between the means of the prediagnostic and postdiagnosis-pretreatment measurements.

**Table VII.** Ultrasound measurements by analysis of variance—groups 1 and 2

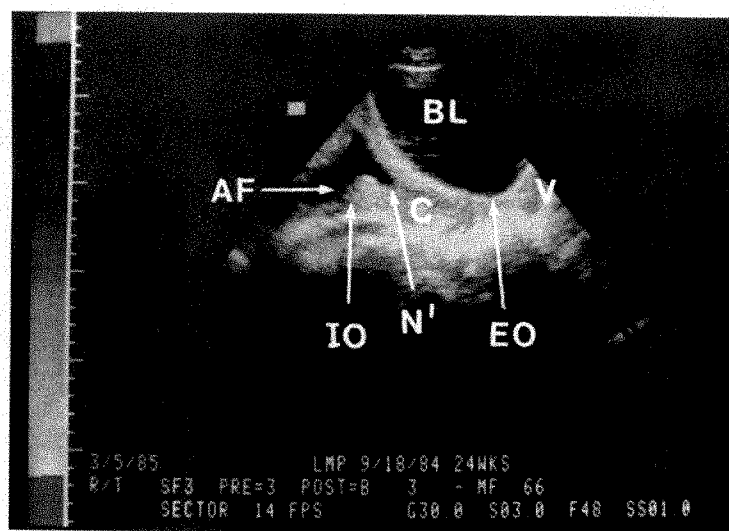
Group	n*	Cervical length (mm)		Membrane protrusion (mm)		Cervical dilatation (mm)		Probability†
		Postdiagnosis-no treatment	Postdiagnosis-posttreatment	Postdiagnosis-no treatment	Postdiagnosis-posttreatment	Postdiagnosis-no treatment	Postdiagnosis-posttreatment	
1	4	32.3 ± 7.3	39.9 ± 12.9	21.3 ± 20.2	5.5 ± 6.4	15.0 ± 13.5	5.3 ± 6.5	0.001
2	18	31.6 ± 12.6	42.0 ± 11.6	18.4 ± 13.9	4.8 ± 5.3	17.6 ± 10.6	4.4 ± 5.0	0.001
Total	22	31.8 ± 11.6	42.2 ± 10.8	18.9 ± 14.7	4.9 ± 5.4	17.1 ± 10.8	4.6 ± 5.2	

\*Not all patients had complete sets of ultrasound data for all contrasts.

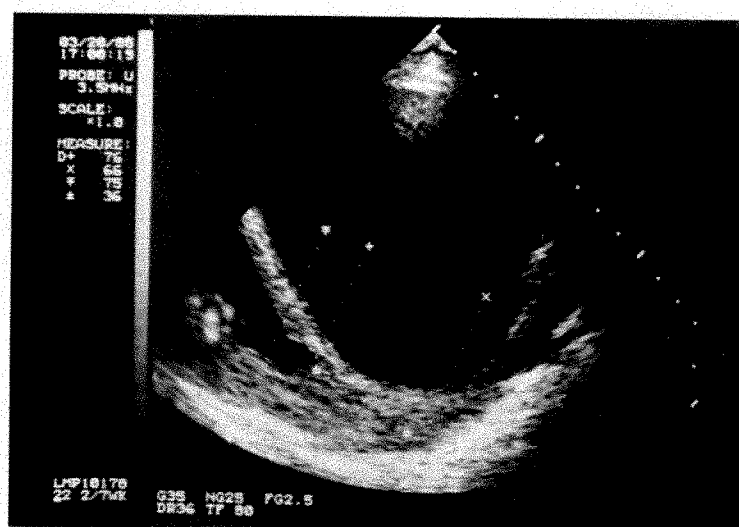
†Probability statements are for the differences between the means of the postdiagnosis-pretreatment and postdiagnosis-posttreatment measurements.

crimutory power of ultrasound as a technique of diagnosis of cervical incompetency, measurements of cervical length, membrane protrusion (nipping and herniation), and dilatation of the cervix were compared before diagnosis and after diagnosis but before treatment for patients found to have cervical incompetency (groups 1, 2, and 3). Groups 4 and 5 were not suited

for this analysis because there were no diagnosed cases. No differences between groups or interaction effects were found in any of the ultrasound variables; however, significant differences were found for the measurements of cervical length, membrane protrusion, and dilatation made before diagnosis and after diagnosis but before treatment ( $p < 0.001$ ) (Table VI).



**Fig. 3.** Early herniation of membranes. *AF* = Amniotic fluid, *C* = cervical stroma, *EO* = estimated plane of sonographic external os, *V* = vagina, *N'* = early herniation, *IO* = sonographic internal os (estimated), and *BL* = bladder.



**Fig. 4.** Advancing membrane protrusion. Patient had frank hourglassed membranes in 48 hours.

**Table VIII.** Ultrasound measurements by analysis of variance —groups 1 and 2

Group	n*	Cervical length (mm)		Membrane protrusion (mm)		Cervical dilatation (mm)		Probability†
		Prediagnosis	Postdiagnosis-posttreatment	Prediagnosis	Postdiagnosis-posttreatment	Prediagnosis	Postdiagnosis-posttreatment	
1	7	43.0 ± 8.5	42.6 ± 12.0	0.0 ± 0.0	3.2 ± 5.4	0.0 ± 0.0	3.0 ± 5.4	NS
2	23	44.8 ± 8.1	41.7 ± 10.8	2.4 ± 3.5	4.7 ± 6.3	1.8 ± 5.5	3.6 ± 4.7	NS
Total	30	44.4 ± 8.1	41.9 ± 10.9	1.8 ± 3.2	4.4 ± 6.1	1.4 ± 4.8	3.4 ± 4.7	NS

\*Not all patients had complete sets of ultrasound data for all contrasts.

†Probability statements are for the differences between the means of the prediagnostic and postdiagnosis-posttreatment measurements.

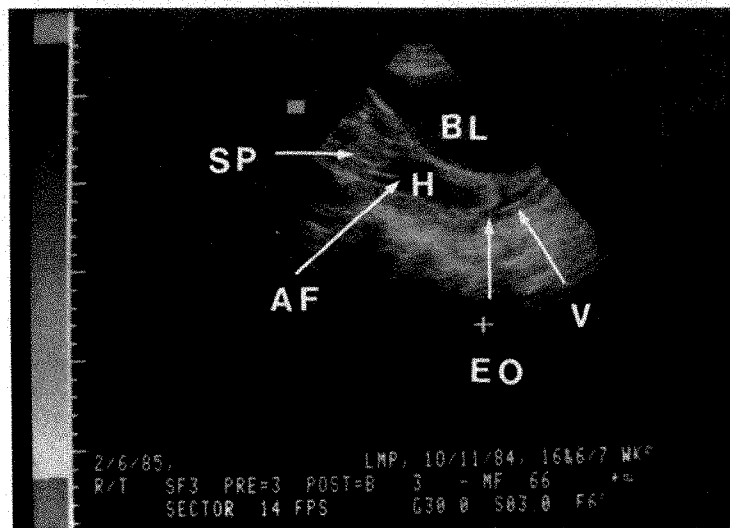


Fig. 5. Advanced herniation. AF = Amniotic fluid, H = advanced herniation, SP = small parts, V = vagina, BL = bladder, and EO = external os.

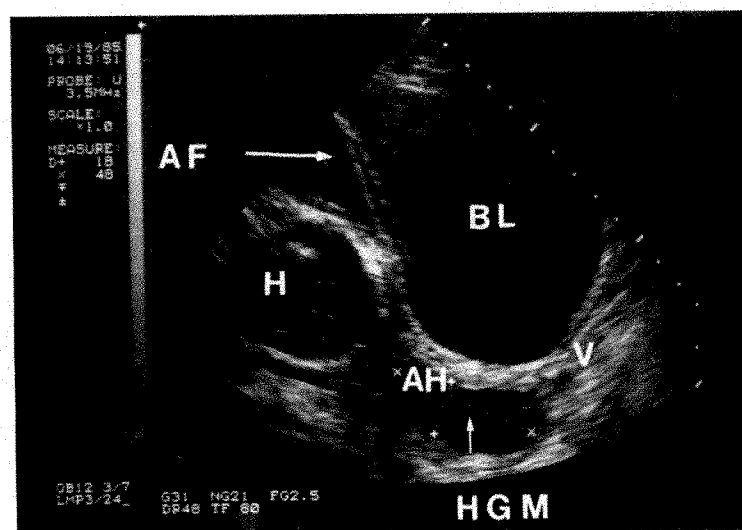


Fig. 6. Frank hourglassing. H = Head, HGM = hourglassing membranes, AF = amniotic fluid, V = vagina, BL = bladder, and AH = advanced herniation.

Measurements of cervical length, membrane protrusion (herniation and nipping), and dilatation of the cervix were contrasted for treated patients (groups 1 and 2) after diagnosis and before treatment as well as after diagnosis and after treatment for patients on whom intervention was possible. As before, no differences between groups or interactions were found on any of the ultrasound variables; however, significant differences in several ultrasound variables were found between the pretreatment and posttreatment measurements. After treatment, cervical length increased significantly ( $p < 0.001$ ). Cervical dilatation and membrane protrusion declined significantly ( $p < 0.001$ ) (Table VII).

Measurements of cervical length, membrane protrusion,

and dilatation were contrasted for treated patients (groups 1 and 2) before diagnosis as well as after diagnosis and after treatment; they demonstrated the effect of treatment on cervical restoration (Table VIII). As before, no differences between groups or interactions were found on any of the ultrasound variables. Importantly, there were no significant differences between prediagnostic measurements and postdiagnostic-posttreatment measurements, which indicates the restorative effect of surgery (Table V).

#### Comment

The failure of the international obstetric community to reduce the 7% incidence of prematurity suggests that innovative protocols need to be developed to reduce

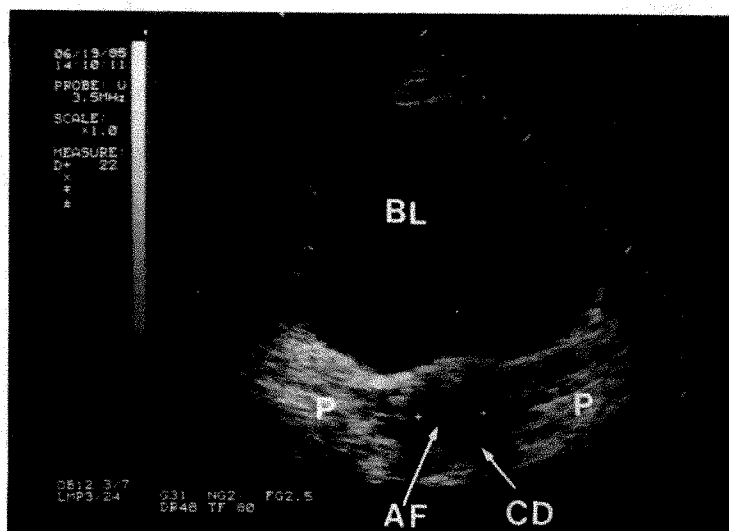


Fig. 7. Advanced cervical dilatation. P = Parametrium base broad ligament, BL = bladder, CD = cervical dilatation, and AF = (arrow) amniotic fluid.

the prematurity rate.<sup>4</sup> It has been suggested that cervical incompetency and its sequelae are responsible for up to 25% of all preterm deliveries. The study demonstrates that the incidence of premature delivery is high when the diagnosis of cervical incompetency is made. While we were unable to confirm an overall decrease in the prematurity rate at our institution, we were impressed by the fact that the incidence of preterm delivery among group 3 patients (diagnosed but not treated) was significantly higher than the incidence of prematurity among groups 2, 4, and 5 ( $\chi^2 = 14.8$ ,  $df = 4$ ,  $p < 0.01$ ). Moreover, patients at risk (groups 1 through 4) were more likely to deliver infants with smaller mean birth weights than were the control patients ( $p < 0.001$ ). It was also equally impressive that group 3 patients diagnosed to have cervical incompetency but not treated had infants with significantly lower mean birth weights than either group 4 or control patients ( $p < 0.001$ ). It appears that when cervical incompetency is excluded by sonographic criteria, prematurity cannot be entirely prevented (group 4). As in the case of cervical incompetency, the etiologies for preterm delivery are not homogeneous. Thirty-two patients (group 2) appear to have been positively assisted by ultrasound criteria for the early diagnosis of cervical incompetency. In each instance, when the decision to treat was made, symptoms or pelvic findings were inconclusive.

The means and standard deviations for sonographic measurements of cervical length and dilatation established by this study compare favorably with those already reported in the literature.<sup>5,6</sup> We are not aware, however, of such means for measurements of membrane protrusion. Nevertheless, although these measurements can be applied to patients at risk for developing cervical incompetency, it appears that each pa-

tient, as demonstrated in this report, must also serve as her own control. Furthermore, the results of each examination must be correlated with clinical findings.

We are impressed with the fact that the development of cervical incompetency is not static but rather a dynamic process. Quite often these changes occur at the level of the internal os and are inaccessible by routine examination. Sonographic changes may be the earliest indicators of incipient cervical failure. Once significant changes are observed, the remaining time to clinical prolapse of the membranes is unpredictable. If suspicious but nondiagnostic changes are noted on the sonogram, the study must be repeated in 24 to 48 hours.

Sonographically observable changes are believed to represent a continuum of pathologic events that should be classifiable by stage and severity. We are now in the process of further analyzing the ultrasound data to develop a weighted stage and grade system to assist in recognizing the earliest signs of cervical incompetency. To further understand the developmental stages of cervical incompetency, we are continually adding patients to our data base. We expect to eventually report on 200 completed cases. This analysis represents preliminary data from the first 85 patients who have completed their pregnancies.

In summary, significant and clinically predictive changes have been demonstrated in the behavior of all measurements believed to be of value in assisting in the sonographic diagnosis of cervical incompetency. We have now accumulated data on at least 32 patients at risk for cervical incompetency for whom the early diagnosis probably would not have been made if sonographic examination had not been done. The most important changes of cervical incompetency are illustrated (Figs. 2 through 7). Alternatively, 51 patients have been excluded from operative treatment by sono-



graphic criteria. Finally, we have demonstrated that significant differences in prediagnostic, postdiagnostic-pretreatment, and postdiagnostic-posttreatment measurements can be demonstrated by ultrasound examination. Successful management of patients at risk in this study was dependent on the combined use of both serial ultrasound and pelvic examinations. The corrected perinatal mortality rate in this study compares favorably with the institutional rate of 15.3 per 1000 live births.

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## Gestational diabetes: Impact of home glucose monitoring on neonatal birth weight

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Two groups of 58 gestational diabetic women matched for age, prepregnancy weight, height, and parity were studied. The home glucose monitoring study group performed fasting and 1-hour postprandial capillary blood glucose testing after every meal. The control group was followed by conventional treatment. The incidence of macrosomia (birth weight of  $\geq 4000$  gm) and large ( $\geq 90\%$ ) for gestational age infants was significantly reduced in the home glucose monitoring group. The mean birth weight of the study group was  $3231 \pm 561$  gm, while that of the control group was  $3597 \pm 721$  gm ( $p < 0.002$ ). Significantly more patients in the home glucose monitoring group were receiving insulin therapy (50% versus 21%). We believe that intensive home glucose monitoring will allow for the early identification of those gestational diabetic patients needing insulin and thus reduce the incidence of macrosomia and large for gestational age infants. (AM J OBSTET GYNECOL 1986;154:546-50.)

**Key words:** Gestational diabetes, home glucose monitoring, macrosomia

Gestational diabetes, defined as carbohydrate intolerance of variable severity with onset or first recognition during the present pregnancy, occurs in approximately 5% of all gravid women.<sup>1</sup> Most studies have shown that these pregnancies have a significant increase in incidence of macrosomia (birth weight  $\geq 4000$  gm)

and large ( $\geq 90\%$ ) for gestational age infants.<sup>1-3</sup> Macrosomia is associated with an increased cesarean section rate and an increased incidence of traumatic vaginal deliveries, which cause significant neonatal morbidity.

Coustan and Imarah<sup>4</sup> have recently reported a significant decrease in macrosomia by administering "prophylactic" insulin to all gestational diabetics. In addition, the rates of operative delivery and birth trauma were significantly reduced. Most centers, however, feel that not all gestational diabetics need insulin therapy and have been reluctant to adopt the prophylactic approach.

Home glucose monitoring by means of capillary glucose determinations have allowed for the intensive management of diabetic patients in an outpatient set-

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ting. Its primary use has been in regulating insulin dosage to achieve tight blood glucose control (that is, near normal fasting and postprandial blood glucose levels). In our study we have tested whether the intensive use of home glucose monitoring would further reduce the incidence of macrosomia and large for gestational age infants. A matched-pair analysis was undertaken to assess neonatal outcome in a group that had received traditional therapy as compared to a group of newly diagnosed gestational diabetic women performing home glucose monitoring.

### Material and methods

The patient population consisted of two groups of 58 pregnant women enrolled in the prenatal diabetes clinic of the Mount Sinai Medical Center in New York City between July, 1979, and July, 1984. Over 90% of the patients seen were black or hispanic and of lower socioeconomic status. Most patients enrolled for prenatal care in the first trimester of pregnancy.

Before 1983 all pregnant patients registered at the Mount Sinai Hospital Prenatal Clinic were screened for glucose intolerance with a 3-hour oral glucose tolerance test if they had one of 10 previously published risk factors for gestational diabetes.<sup>5</sup> After 1983 all patients at the time of enrollment in the prenatal clinic were given a random 50 gm oral glucose screening. Patients with an oral glucose plasma value of  $\geq 135$  mg/dl after 1 hour underwent a full 100 gm oral glucose tolerance test. The diagnosis of glucose intolerance was based on the criteria of O'Sullivan and Mahan<sup>6</sup> modified to correct for the methodologic change from the Somogyi-Nelson method to glucose oxidase and for measurement of plasma rather than whole blood glucose.<sup>7</sup> The diagnosis of gestational diabetes was made when two values met or exceeded the following criteria: fasting = 95 mg/dl, 1 hour = 180 mg/dl, 2 hour = 155 mg/dl, or 3 hour = 135 mg/dl. After the diagnosis of gestational diabetes was made, all patients were referred to the prenatal diabetes clinic and started on a diabetic diet (30 to 35 kcal/kg of ideal body weight and consisting of 25% fat, 25% protein, and 50% complex carbohydrate). Patients were seen weekly in the clinic, at which time a 2-hour postprandial capillary blood glucose measurement was performed.

The index group was enrolled beginning in September, 1983, after which time all patients, including those on only diet management were started on home glucose monitoring. Fasting and 1-hour postprandial values were obtained daily by the patient with use of a visually read Chemstrip bG glucose test (Bio-Dynamics, Indianapolis, Indiana). Values were verified weekly by rechecking the strips with a reflectance meter in the clinic, which revealed close correlation between visual and reflectance values. Insulin therapy was begun if fasting

**Table I.** Demographic data of group with home glucose monitoring and control group

	Home glucose monitoring	Control	Probability
No. of patients	58	58	
Age (yr)	30.4 $\pm$ 6	30.1 $\pm$ 6	NS
No. of nulliparas	18	14	NS
% of nulliparas	31	24	
Percent prepregnancy ideal body weight	127 $\pm$ 28	123 $\pm$ 26	NS
Hispanic (%)	64	59	NS
Black (%)	33	34	NS

NS = Not significant.

glucose values were  $>95$  mg/dl or if postprandial values were  $>120$  mg/dl, with dosages adjusted to maintain values below these levels. All patients except those registering after 36 weeks were enrolled in the study. Patient compliance with performance of home glucose monitoring was  $>90\%$ .

Patients observed before September, 1983, were used as case control subjects to assess the impact of home glucose monitoring on neonatal outcome. Since the screening procedure and criteria for the diagnosis of glucose intolerance in pregnancy varied during this time period, only those patients fitting the standard glucose tolerance test criteria listed above were retrospectively selected. The patients thus identified were then randomly listed by computer and matched with the study group by a scan down the list until an appropriate control was identified. Patients were matched for age, prepregnancy weight, height, ideal body weight, and parity (primiparas or multiparas). These women received precisely the same care as the study group except they did not use home glucose monitoring when on diet therapy only. Insulin therapy was started for these patients when weekly 2-hour postprandial capillary glucose values obtained in the clinic were  $>120$  mg/dl.

Neonatal data were obtained from neonatal charts. Gestational age was assigned with use of the last menstrual period and sonographic dating criteria. Dubowitz scoring was used for confirmation of the gestational age, which was used in the calculation of birth weight ratios (birth weight divided by mean birth weight for gestational age).

Statistical analysis was performed with use of a two-tailed *t* test and McNemar's test to assess significance.<sup>8</sup>

### Results

No significant differences in demographic characteristics were noted between the two groups (Table I). The mean age, percentage of nulliparous women, and racial percentages were not significantly different in the two groups. Percent prepregnancy ideal body weight,

**Table II.** Neonatal outcome of two groups

	Home glucose monitoring	Control	Probability
Birth weight (gm)	3231 $\pm$ 561	3597 $\pm$ 721	$p < 0.002$
Macrosomia ( $\geq 4000$ gm) (n)	5 (9%)	14 (24%)	$p < 0.05$
Large for gestational age (n)	7 (12%)	24 (41%)	$p < 0.005$
Birth weight ratio	1.06 $\pm$ 0.15	1.18 $\pm$ 0.21	$p < 0.001$

as determined from Metropolitan Life Height and Weight Tables (1983), was also not statistically different.

There was a significant difference in the use of insulin in the two groups. Twenty-nine (50%) patients in the home glucose monitoring group were treated with insulin as compared to 12 (21%) patients in the control group ( $p < 0.01$ ). However, the duration of insulin therapy was not significantly different in the two groups ( $9.8 \pm 7$  weeks and  $8.5 \pm 7$  weeks in the home glucose monitoring and control groups, respectively;  $p > 0.05$ ). In addition, no significant difference was found in the week of gestation that insulin therapy was started in the home glucose monitoring group ( $29 \pm 7$  weeks) as compared to the control group ( $31 \pm 8$  weeks).

The spontaneous vaginal delivery rate was 47% in the home glucose monitoring group compared to 65% in the control group. The forceps rate was 21% in the home glucose monitoring group and 10% in the control group. Cesarean sections were performed in 32% of the home glucose monitoring cases and in 25% of the control cases. None of these differences were statistically significant.

Significant differences were observed in birth weights in the two groups, as noted in Table II. The mean birth weight was  $3231 \pm 561$  gm in the home glucose monitoring group and  $3597 \pm 721$  gm in the control group ( $p < 0.002$ ). The incidence of macrosomia ( $\geq 4000$  gm) was also significantly reduced in the home glucose monitoring group, being 9% compared to 24% ( $p < 0.05$ ). In addition, there were significantly fewer large ( $\geq 90\%$ ) for gestational age infants in the home glucose monitoring group, or 12% compared to 41% ( $p < 0.005$ ). The birth weight ratio in the home glucose monitoring group ( $1.06 \pm 0.15$ ) was significantly less than in the control group ( $1.18 \pm 0.21$ ) ( $p < 0.001$ ).

Possible confounding factors were considered in the two groups. The gestational age at birth was not significantly different in the two groups ( $38.8 \pm 2$  weeks in the home glucose monitoring group compared to  $39.1 \pm 2$  weeks in the control group). The gestational age at the time of the diagnosis was also not significantly different in the two groups ( $26.8 \pm 7$  weeks in the

**Table III.** Oral glucose tolerance test plasma values

	Home glucose monitoring	Control	Probability
Fasting (mg/dl)	98 $\pm$ 17	104 $\pm$ 16	$p < 0.05$
1 hour (mg/dl)	206 $\pm$ 41	200 $\pm$ 37	NS
2 hour (mg/dl)	182 $\pm$ 43	177 $\pm$ 45	NS
3 hour (mg/dl)	138 $\pm$ 44	127 $\pm$ 42	NS

home glucose monitoring group compared to  $29.1 \pm 7$  weeks in the control group). To try to assess the relative degree of glucose intolerance in the two groups, individual values on the initial glucose tolerance test were compared between the groups (Table III). The only significant difference was found in the fasting value, which was significantly increased in the control group ( $103 \pm 16$  compared to  $98 \pm 17$ ) ( $p < 0.05$ ). To further assess this relationship, the fasting values of the large for gestational age infants in the home glucose monitoring and control groups combined were compared to those of the average for gestational age babies in both groups. There was a significantly higher fasting value noted in the large for gestational age group ( $107 \pm 14$ ) as compared to the average for gestational age group ( $98 \pm 17$ ;  $p < 0.01$ ). However, an abnormal fasting value on the oral glucose tolerance test ( $\geq 95$  mg/dl) was not found to be predictive of large for gestational age or macrosomic infants. Although 85% of large for gestational age and macrosomic infants had an elevated fasting value, 55% of average for gestational age infants also had an elevated value. In addition, 50% of the women in the home glucose monitoring group requiring insulin therapy had fasting plasma glucose values of  $< 105$  mg/dl.

Because all patients on home glucose monitoring also had a 2-hour postprandial capillary glucose test performed at each clinic visit, it was possible to compare these values between the two groups. There was no significant difference noted in the clinic postprandial value in the home glucose monitoring group ( $110 \pm 18$  mg/dl) as compared to the control group ( $102 \pm 17$  mg/dl).

### Comment

Modern management of gestational diabetes has reduced the rate of perinatal mortality to that of the general population.<sup>1</sup> However, significant perinatal morbidity continues to occur, much of it related to the increased incidence of macrosomia in this population. Most authors report an incidence of 15% to 30% of macrosomia in "well-controlled" gestational diabetic patients,<sup>1-3</sup> implying that other metabolic fuels may be important in causing macrosomia.

Despite this, the Pedersen hypothesis that maternal hyperglycemia causes fetal hyperglycemia and hyper-

insulinemia leading to increased growth remains attractive.<sup>9</sup> Initial studies by O'Sullivan et al.<sup>10</sup> in 1974 and Coustan and Lewis<sup>11</sup> in 1978 demonstrated a significant decrease in macrosomia in gestational diabetic women given prophylactic insulin. Roversi et al.<sup>12</sup> in 1980 were able to reduce the incidence of macrosomia to 2.6% in a group of gestational diabetic women who were treated with maximally tolerated doses of insulin. However, average 24-hour blood glucose levels were 90 mg/dl before therapy and 70 mg/dl after therapy. In 1984 Coustan and Imarah achieved an incidence of macrosomia of 7% in a group of gestational diabetic women prophylactically given a dose of 20 units of NPH insulin and 10 units of regular insulin.<sup>4</sup> They also found that the degree of initial glucose intolerance on the oral glucose tolerance test was not predictive of the level of macrosomia for women treated by this protocol. Thus while the reduced incidence of macrosomia in both studies appeared to be related to aggressive insulin therapy, the relationship between outcome and the degree of maternal glucose intolerance was unclear. Specifically, it was not clear whether an equally favorable outcome would have been possible without treating all gestational diabetic women with insulin. Also, we questioned whether an average blood glucose level as low as 70 mg/dl is necessary to prevent macrosomia. In addition, there is potential risk to insulin treatment, including hypoglycemia and insulin antibody production. Because of these concerns, we instituted home glucose monitoring for all gestational diabetic women to assess more accurately the glucose levels in these patients throughout the day.

Home glucose monitoring has resulted in daily assessment of outpatient glucose control in our clinic population. Patient compliance was extremely high, with over 90% of patients performing daily glucose monitoring four times each day. By asking all of our patients to save their strips in a sealed container and having them verified weekly, we were able to obtain reliable values and promote compliance. This intensive approach of measuring daily fasting and 1-hour postprandial values has allowed us to identify an increased number of patients needing insulin therapy to maintain relative normoglycemia. The incidence of macrosomia (9%) and large for gestational age babies (12%) and the birth weight ratios (1.06) were all significantly reduced in the study group as compared to the group of matched control subjects who did not use home glucose monitoring.

Standard management in most centers consists of once- or twice-weekly postprandial glucose measurements in gestational diabetic women receiving diet management. In our study a comparison of the mean clinic postprandial values in the home glucose monitoring and control groups revealed no significant dif-

ferences. Thus we feel that the clinic postprandial glucose determinations are not sensitive enough in predicting which patients need insulin therapy. In questioning patients we have found that some patients alter their diet on their clinic days so that they have a "good" value.

The association between an elevated fasting plasma glucose value on the oral glucose tolerance test and an increased incidence of macrosomia has been reported by others. Metzger et al.<sup>13</sup> have treated all gestational diabetic women with fasting plasma values of >105 mg/dl with insulin and have shown a significant reduction in macrosomia. Although the mean fasting plasma glucose level was higher in the mothers of large for gestational age infants as compared to average for gestational age infants in our series, not all patients with elevated fasting values on the glucose tolerance test required insulin therapy. In addition, of the women in the home glucose monitoring group requiring insulin therapy, 50% had fasting plasma glucose values of <105 mg/dl. Thus it appears that home glucose monitoring is a more sensitive and specific means of determining the need for insulin therapy than use of a single fasting glucose value.

With the institution of home glucose monitoring we have found that 50% of our gestational diabetic women required insulin therapy. Although precisely the same criteria were used in the decision to start insulin therapy in both home glucose monitoring and control groups (that is, fasting blood glucose >95 or postprandial glucose >120), a significantly greater number of patients in the home glucose monitoring group were identified as needing insulin. Home glucose monitoring and selective insulin therapy have made it possible to achieve an overall incidence of macrosomia equal to that achieved with prophylactic insulin treatment of all gestational diabetic women. Thus we recommend the use of home glucose monitoring for all gestational diabetic patients to individually tailor their management and to identify those needing insulin therapy.

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## An obstetric assessment of the first 100 births from the in vitro fertilization program at Clamart, France

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From April, 1981, to July, 1984, 142 pregnancies have been attained after in vitro fertilization and embryo transfer. They are divided into 22 biochemical pregnancies (human chorionic gonadotropin  $\geq 20$  mU/ml but remaining below 1000 mU/ml), 27 spontaneous abortions, three ectopic pregnancies, and 90 ongoing pregnancies, of which 11 were twin pregnancies. The 90 women in whom the pregnancies progressed were compared with the 52 women having nonprogressive pregnancies. The two populations did not differ either in age, in the indication for in vitro fertilization and embryo transfer, or in the quality of ovulation or results of semen analysis. The 90 ongoing pregnancies were compared with those pregnancies occurring in the same obstetrics department during this period. We found that the in vitro fertilization group had a higher proportion of arterial hypertension (16.5% versus 8.5%,  $p < 0.05$ ), breech presentations (13.9% versus 4.3%,  $p < 0.001$ ), and caesarean sections (46.8% versus 15.5%,  $p < 0.001$ ) but the sex ratio did not differ. (*AM J OBSTET GYNECOL* 1986;154:550-5.)

**Key words:** In vitro fertilization and embryo transfer, obstetric outcome

After the success of Edwards and Steptoe,<sup>1</sup> the procedure of in vitro fertilization and embryo transfer has become one of the possible treatments for certain types of infertility. More than 2000 children have been conceived by this method. Little information is available on the outcome of these pregnancies as the patients are not usually delivered in the in vitro fertilization center. At Clamart, the in vitro fertilization center is part of the obstetrics department and this enables us to follow up most of the pregnancies and to establish a register of both the pregnancies and the births for all patients. We present in this paper an assessment of the first 142 pregnancies. We have compared the spontaneous abor-

tion rate, fetal growth, obstetric complications, and method of delivery in these pregnancies with those occurring after spontaneous pregnancies as well as after induction of ovulation.

### Patients and methods

From April, 1981, when the first in vitro pregnancy occurred—which ended as a spontaneous abortion<sup>2</sup>—to July, 1984, when the one hundredth baby was conceived, 1280 oocyte retrievals were performed for in vitro fertilization and 142 pregnancies commenced. All patients included in the in vitro fertilization program had a preliminary workup comprising an assessment of ovulation, a semen analysis, and an assessment of previous surgical procedures to decide whether they needed a laparoscopic examination. All 142 pregnancies occurred after ovulation induction. A combination of clomiphene citrate (Merrell Torraude, Paris, France) and human menopausal gonadotropins (Inductor, Searle, Paris, France) was used in 94% of the

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**Table I.** Population analysis

	Ongoing pregnancies	Nonprogressive pregnancies
No.	90	52
Age (yr)	32.1 ± 3.3	32.8 ± 3.9
Sterility (%)		
Primary	44	39
Secondary	56	61
Indication for in vitro fertilization (%)		
Tubal sterility	92	88
Immunologic	4.5	1.9
Idiopathic	1.1	3.8
Normal ovulation (%)	58.2	55.3
Normal semen analysis (%)	91.3	85.2

cases. In all but four cases human chorionic gonadotropin (hCG) was given in lieu of the ovulatory surge and oocyte recovery took place  $35 \pm 1$  hours afterward, either laparoscopically with the patient under general or local anaesthesia or with ultrasonographic guidance according to previously described techniques.<sup>3</sup> The details of ovulation monitoring and of in vitro culture techniques have been described elsewhere.<sup>3,4</sup> After embryo transfer the only treatment given was dydrogesterone (Duphaston, Duphar, Villeurbanne, France) in known cases of luteal insufficiency.

Commencing on the eleventh day after oocyte aspiration (13 days after the administration of hCG) the serum levels of hCG were measured by radioimmunoassay every 2 days. The assays were repeated every 2 days until the twenty-third day, then weekly for the first 10 weeks of amenorrhea. Ultrasonography was performed around the eighth week of amenorrhea. We defined biochemical pregnancy by an hCG value  $\geq 20$  mIU/ml on two occasions after the eleventh day following aspiration but never exceeding 1000 mIU/ml.<sup>5</sup> In fact when this last value was reached, a gestational sac was always detected by the ultrasound scan and the pregnancy had become clinical. Should the pregnancy then not proceed, we termed it a spontaneous abortion. This classification of pregnancy is similar to that of Edwards and Steptoe<sup>6</sup> but differs from that of Jones et al.,<sup>7</sup> as we found that with the use of clomiphene and human menopausal gonadotropins, 50% of the luteal phases exceed 15 days in the absence of a pregnancy.<sup>5</sup>

The 142 pregnancies were divided in the following way: A total of 90 pregnancies progressed to term, and among these there were 11 twin pregnancies and 79 singleton pregnancies resulting in 100 living babies and one intrauterine death. There were 22 biochemical pregnancies, and 27 pregnancies terminated as spontaneous abortions. Three ectopic pregnancies occurred during this period.

First of all we have compared the ongoing pregnan-

**Table II.** Number of embryos transferred and the progress of the 142 pregnancies

	No. of embryos transferred		
	1	2	3
Total No. transferred	50	57	35
Ongoing pregnancies (% of all pregnancies)	66	60	66
Twin pregnancies (% of ongoing pregnancies)	0	21	22

cies with those that failed to progress; then we have compared the in vitro pregnancies with those obtained after induction of ovulation as well as with all pregnancies with delivery at Antoine Bécclère Hospital during the same period. We looked at the singleton and multiple pregnancies separately.

**Statistical method.** Sample means were compared by means of Student's *t* tests and proportions compared by  $\chi^2$  analysis. Means are expressed together with the standard deviation.

## Results

### Comparison between ongoing and nonprogressive in vitro pregnancies

*Population analysis (Table I).* The proportion of patients with primary or secondary sterility and the indication for in vitro fertilization did not differ between the two groups. Ninety percent of patients in the program were candidates for in vitro fertilization on account of tubal sterility. Similarly the percentages with normal ovulation and normal results of semen analysis did not differ. Mean age of patients who aborted (32.8 years) was the same as that of those in whom pregnancy progressed (32.1 years). The proportions of laparoscopic oocyte recovery of ultrasonically guided puncture were the same in the two groups (81% and 82% laparoscopic oocyte recovery).

*Biochemical pregnancies.* The level of hCG remained below 300 mIU/ml in 20 of the 22 cases and between 300 and 1000 in two cases. In 18 cases the hCG threshold<sup>5</sup> appeared more than 13 days after follicular puncture and there was an inadequate rise from one measurement to the other.<sup>8</sup> The duration of the luteal phase had been prolonged from 0 to 15 days with a mean of 5 days.

*Spontaneous abortions.* These occurred most often between the sixth and twelfth weeks of amenorrhea (25 cases). The expulsion of the fetus was always delayed in relation to the fall in hCG, even necessitating an aspiration of the uterine cavity in five cases where the fetus had not been expelled 1 month after the pregnancy terminated. In the 27 cases, only six chromosomal analyses could be performed. Two were normal,

**Table III.** Analysis of the three pregnancy groups (excluding twins)

	<i>Spontaneous (control) pregnancies</i>	<i>In vitro fertilization pregnancies</i>	<i>Induced ovulation pregnancies</i>
No.	3841	79	142*
Age (yr)	28.6 ± 4.2*	32.5 ± 3.5	29.3 ± 4.2
Primigravid (%)	46.2*	83.5	57.8
No. of consultations	6.9 ± 3.7*	8.3 ± 2.1	6.8 ± 1.6
Weight gain (kg)	12.8 ± 3.7	12.9 ± 3.6	12.6 ± 3.6

Mean ± SD.

\*p &lt; 0.001.

**Table IV.** Pregnancy complications (excluding twins)

	<i>Spontaneous (control) pregnancies</i>	<i>In vitro fertilization pregnancies</i>	<i>Induced ovulation pregnancies</i>
No.	3841	79	142
First-trimester bleeding			
No.	374	12	19
% All pregnancies	9.7	15.2	13.4
Threatened premature delivery			
No.	420	6	23
% All pregnancies	10.9	7.6	16.2
Arterial hypertension			
No.	327*	13	13
% All pregnancies	8.5	16.5	9.1
Fetal growth retardation			
No.	443	7	21
% All pregnancies	11.5	14.8	14.8

\*p &lt; 0.05.

one revealed a monosomy 45,X, and the other three showed trisomies 20, 22, and 15. Only two abortions were recorded later than 12 weeks, one in a twin pregnancy at 17 weeks and the other at 18 weeks associated with a pyrexia after amniocentesis.

**Ectopic pregnancies.** Among the three ectopic pregnancies, two were easily diagnosed at 7 weeks of amenorrhea by ultrasound and the levels of hCG. These were ampullary ectopic pregnancies in patients who had a past history of tubal surgical procedures and who were known to have hydrosalpinges. The third was a pregnancy implanted in the right uterine cornu in a woman who had previously undergone a right salpingectomy for an ectopic pregnancy. The diagnosis was made only at 10 weeks of amenorrhea when she presented with the signs of a hemoperitoneum.

**Number of embryos transferred (Table II).** When a pregnancy occurred, whatever the number of embryos transferred, the percentage of ongoing pregnancies was around 60. The percentage of twin pregnancies was virtually the same whether two or three embryos were transferred (21 and 22). The 11 sets of twins developed from the transfer of two embryos in six cases and three embryos in five cases. Of the 27 abortions, two occurred in the course of a known twin pregnancy (after the transfer of two embryos in both cases). However, as the first ultrasound examination was requested

only after 8 weeks of amenorrhea, there must be a certain number of early twin pregnancies, of which we are unaware, that subsequently become singleton gestations.

#### **Comparison of the singleton in vitro ongoing pregnancies (n = 79) with the induced (n = 142) and control (n = 3841) pregnancies**

**Age, parity, and antenatal progress (Table III).** The patients who had successful in vitro fertilization were older than the women with induction of labor and those with spontaneous pregnancies (p < 0.001). The proportion of primigravid women in the in vitro fertilization group was higher than in the other two groups (p < 0.001). The number of antenatal consultations was much greater in the in vitro fertilization patients but the weight gain was identical in all three groups. The first 10 patients who became pregnant after in vitro fertilization systematically underwent amniocentesis, but in the remainder only those patients in whom amniocentesis was justified on account of their age or past obstetric history were submitted to it.<sup>9</sup>

**Pregnancy complications (Table IV).** We limited ourselves to studying bleeding in the first trimester, threatened premature delivery justifying hospitalization, fetal growth retardation, and hypertension requiring treatment. The percentage of threatened premature deliveries tended to be lower in the in vitro pregnancies, as

**Table V.** Outcome of deliveries

	<i>Spontaneous (control) pregnancies</i>	<i>In vitro fertilization pregnancies</i>	<i>Induced ovulation pregnancies</i>
No.	3841	79	142
Premature deliveries			
No.	144	5	13
% All deliveries	3.7	6.3	9.1
Cesarean sections			
No.	597	37	31
% All deliveries	15.5*	46.8*	21.8
Breech presentations			
No.	166	11	8
% All deliveries	4.3*	13.9*	5.6
Sex ratio			
Girls (%)	46.9	55.7	46.5
Boys (%)	53.1	44.3	53.5
Birth weight (gm)	3273 ± 490	3249 ± 460	3142 ± 557

\* $p < 0.001$ .

**Table VI.** Twin pregnancies

	<i>Spontaneous (control) pregnancies</i>	<i>In vitro fertilization pregnancies</i>	<i>Induced ovulation pregnancies</i>
Total No. of pregnancies	3899	90	163
No. of twin pregnancies	58	11	21
Frequency of twin pregnancy (%)	1.5	12.2	12.8
Cesarean sections			
No.	26	8	10
% All deliveries	44.8	72.7	47.6

might be expected because these women were immediately advised to rest. This difference did not reach significance. The percentage of women presenting with hypertension requiring treatment in the third trimester was higher in the in vitro fertilization group ( $p < 0.05$ ). Similarly we noted an increase in first-trimester bleeding in patients after induction of ovulation (NS).

**Deliveries (Table V).** The percentage of premature deliveries (6.3) was the same as in the control population (3.7). Paradoxically the highest proportion of premature deliveries was seen among the in vivo pregnancies after induction of ovulation. The percentage of cesarean sections increased from 15.5% in the control group to 21.5% in the in vivo pregnancies after induction of ovulation and to 48.6% in the in vitro pregnancies ( $p < 0.001$ ). Elective procedures represented 40% of the cesarean sections in the in vitro fertilization group versus 32% in the pregnancies after induced ovulation and 41% in the control group. Breech presentations were significantly more frequent in the in vitro pregnancies (13.9% versus 4.3% in the control group and 5.6% among the induced ovulation pregnancies,  $p < 0.001$ ). On the other hand the birth weights and the sex ratios were very similar in the three groups. Fifty-two of the 79 placentas were subjected to histopathologic examination but no abnormalities were demonstrated. Among the 79 singleton pregnancies there was one unexplained intrauterine death at term.

This involved a macrosomic infant (weight of 4700 gm and 61 cm in length) whose mother had no relevant past history or premonitory signs and in whom the autopsy gave no explanation of the cause of death. Among the 78 living children one was subsequently discovered to have a small diaphragmatic hernia, which was successfully repaired.

**Twin pregnancies ( $n = 11$ ) (Table VI).** Twin gestations represented 11 of the 90 pregnancies for a frequency of 12.2%. The frequency of twins was 12.8% in the induced pregnancies and 1.5% in the spontaneous pregnancies. The antenatal course of the in vitro twin pregnancies did not differ from that in those occurring after ovulation induction. The frequency of cesarean section in the in vitro fertilization group was increased compared to that in the other two groups but the difference was not significant. In all 11 cases the twins were dichorionic and diamniotic.

### Comment

An increased percentage of spontaneous abortions and biochemical pregnancies (34.5% of the pregnancies conceived in our unit) was found in all series.<sup>10</sup> Is this increase due to the technique of in vitro fertilization? Candidates for in vitro fertilization are infertile women who are already more predisposed than other women to spontaneous abortions and ectopic pregnancies. The risk of spontaneous abortion was estimated to be 10%



to 15% in the normal population<sup>11</sup> and 21.7% to 30.5% among infertile women, depending on the series.<sup>12</sup> Furthermore, all in vitro fertilization patients undergo ovarian stimulation and, while the frequency of spontaneous abortion after ovulation induction varies from one treatment regimen to another, it is always in the region of 20%.<sup>11</sup> If we exclude the biochemical pregnancies (as they are usually undetected) from our study, the percentage of clinical spontaneous abortions was 22.5%, which is not very different from the aforementioned figures. Finally, Edmonds et al.<sup>13</sup> have shown that in 34% of ovulatory cycles serum hCG reveals the presence of an embryo during the luteal phase that is subsequently lost before the pregnancy has a chance to become clinically apparent. Most of the time it is possible to predict immediately that the pregnancy will not progress and will remain a biochemical one by means of the first two hCG assays.<sup>8</sup>

The percentage of ectopic pregnancies after in vitro fertilization, which reaches 11% of pregnancies in some series,<sup>14</sup> is cause for concern. The ectopic pregnancies are more likely to be due to eggs that are initially placed in the uterus and move up toward the tube rather than to the untoward placing of the egg in the tubal ostium. This interpretation is suggested by the occurrence of bilateral ectopic pregnancies after transfer of multiple embryos<sup>15</sup> and the association of intrauterine and extrauterine pregnancies.<sup>16</sup> Their prevention currently seems impossible because bilateral salpingectomy, which could not really be contemplated where sterility is of a nontubal origin, would not solve the problem. There would remain a risk of interstitial ectopic pregnancy or of rupture of the cornu of a scarred uterus if the interstitial portion of the tube were resected.

The increase in the pregnancy rate with the number of embryos transferred is universally acknowledged but is subject to variable mathematical laws.<sup>10, 17</sup> On the other hand, the importance of the number of embryos transferred in relation to the progress of pregnancy has been discussed. The increased risk of abortion described by Edwards and Steptoe<sup>6</sup> is refuted by Muashers et al.<sup>18</sup> There is no consensus on the maximum number of embryos to be replaced. Some would replace up to six,<sup>18</sup> but our position is to restrict replacement to three to limit the number of multiple pregnancies. The percentage of twin pregnancies in our program is 12%. Of the pregnancies resulting from the transfer of two or three embryos only 20% were twin pregnancies. The reason for this is probably that all embryos transferred are not of the same quality.<sup>18</sup> Better control of embryo freezing<sup>19</sup> would permit a reduction in the number of embryos replaced and in the incidence of multiple pregnancy. Although the majority of twin pregnancies after in vitro fertilization are dizygotic ones due to mul-

iple embryo transfer, some monozygotic pregnancies have been described.<sup>20</sup>

The frequency of first-trimester bleeding in induced ovulation and in vitro pregnancies can perhaps be explained by early abortion from an undetected multiple pregnancy. However, this explanation does not take account the bleeding that often occurs after the transfer of a single embryo in pregnant women. The frequency of hypertension can perhaps be explained by the greater number of primigravid patients and the older maternal age in the in vitro fertilization group. Weight gain, however, did not differ between the groups. The increased frequency of toxemia sometimes reported in induced ovulation pregnancies has been confirmed only in the in vitro fertilization group. Intrauterine development is normal after ovulation induction with human menopausal gonadotropins or clomiphene citrate<sup>21</sup> but it has not been well studied after in vitro fertilization.<sup>22</sup>

Fertile women often have an ambivalence between the desire for pregnancy and the desire for children. This invests in vitro fertilization with a special significance because an additional factor among infertile women is the pursuit of their female identity, which has been called into question by their sterility. We were surprised that in many cases women failed to follow the medical advice that was appropriate for this type of precious pregnancy.<sup>23</sup>

The occurrence of malformation appears rare; one cardiac malformation has been described<sup>22</sup> and a single trisomy 21 has been reported in the literature in 600 births.<sup>24</sup> The fetal malformation rate after ovarian stimulation is usually accepted to be 1.8%, which is comparable to the rate observed in spontaneous pregnancy.<sup>25, 26</sup> Is the use of repeated ultrasound, for monitoring ovulation and more recently for the recovery of the oocyte, harmless? Desmoulin et al.<sup>27</sup> found a decrease in fertility in women having artificial insemination who underwent ultrasound monitoring when compared with those who did not. Puissant et al.<sup>28</sup> have shown that ultrasound treatment applied to mouse oocytes before fertilization results in delayed deleterious effects; more resorptions occurred after implantation among the group originating from sonicated oocytes. However, initial results show that ultrasound-guided puncture has a normal ongoing pregnancy rate identical to that of laparoscopic recovery.<sup>3</sup> We cannot explain the significant proportion of breech presentations. The only causal factor noted was the high frequency of primigravid patients and their age. It is not too surprising that a large proportion of deliveries were accomplished by cesarean section, and this phenomenon also occurs in other units<sup>22, 24</sup> partly because of anxiety on the part of the obstetrician when faced with

this rare situation but also perhaps because of the raised proportion of breech presentations. After ovarian stimulation a predominance of female infants has been reported on several occasions,<sup>29-31</sup> but this has not been described in in vitro fertilization.

In conclusion, the percentage of spontaneous abortions after in vitro fertilization is apparently raised only if one takes into account the population for which in vitro fertilization is intended and the ovulation induction treatments used. This raised percentage is explicable without calling into question the technique of in vitro fertilization itself. The occurrence of ectopic pregnancies after in vitro fertilization is no longer surprising. With the exception of maternal hypertension, there are no more complications of ongoing pregnancies after in vitro fertilization than there are after induced ovulation pregnancies or spontaneous pregnancies but the frequency of breech presentation is high. The considerable proportion of cesarean deliveries will undoubtedly lessen as in vitro fertilization pregnancies become more commonplace.

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# Etiology of cervical inflammation

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We studied the relationships of selected microbial, clinical, demographic, and behavioral variables to mucopurulent cervicitis in two clinical settings, a sexually transmitted disease clinic and a student health clinic. From each clinic, we studied a group of women referred for suspected mucopurulent cervicitis and a representative sample of other women attending the clinic. After the women were stratified by patient group and summary odds ratios for all groups were obtained, mucopurulent cervicitis was most strongly associated with the isolation of *Chlamydia trachomatis*; other variables associated with mucopurulent cervicitis included the isolation of *Ureaplasma urealyticum*, *Gardnerella vaginalis*, and *Trichomonas vaginalis*, the presence of serum antibody to *C. trachomatis*, the clinical diagnosis of bacterial vaginosis, and oral contraceptive use (positive associations) or isolation of yeast (negative association). After adjustment for cervical culture results for *C. trachomatis*, mucopurulent cervicitis was positively associated with oral contraceptive use ( $p = 0.02$ ) and isolation of *U. urealyticum* ( $p = 0.02$ ) and negatively associated with isolation of yeast ( $p = 0.03$ ). Among women with a positive cervical culture for *C. trachomatis*, isolation of *U. urealyticum* was significantly associated with mucopurulent cervicitis, while among the subgroup of women with a negative cervical culture for *C. trachomatis* and positive serum antibody to *C. trachomatis*, oral contraceptive use was strongly associated with mucopurulent cervicitis. These results confirm that in both clinical settings *C. trachomatis* is the major cause of mucopurulent cervicitis. The roles of *U. urealyticum*, *T. vaginalis*, *G. vaginalis*, bacterial vaginosis, and oral contraceptive use in the etiology of mucopurulent cervicitis deserve further study. (AM J OBSTET GYNECOL 1986;154:556-64.)

**Key words:** Cervicitis, cervical inflammation, chlamydia, ureaplasma, bacterial vaginosis

Infection of the uterine cervix is often asymptomatic and represents a reservoir for sexual and perinatal transmission of pathogenic microorganisms. It might lead to at least three possible types of complications: (1) ascending intraluminal spread of pathogenic organisms from the cervix, producing endometritis and salpingitis; (2) ascending infection during pregnancy, resulting in chorioamnionitis, premature rupture of the membranes, amniotic fluid infection, premature delivery, and puerperal and neonatal infections; and (3) the initiation or promotion of cervical neoplasia.

In a previous study of randomly selected women attending a clinic for sexually transmitted diseases, we assessed objective criteria for the clinical diagnosis of mucopurulent cervicitis, that is, visualization of yellow

mucopurulent endocervical discharge on a white swab or the presence of 10 or more polymorphonuclear leukocytes per microscopic field (at a magnification of 1000) in satisfactory Gram-stained endocervical smears.<sup>1</sup> These criteria are quite similar to those commonly used to make a presumptive diagnosis of nongonococcal urethritis in men.<sup>2</sup>

The major recognized infectious causes of cervicitis are thought to be the sexually transmitted pathogens *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and herpes simplex virus. However, previous studies of the etiology of cervicitis have focused on selected groups of patients seen in sexually transmitted disease clinics (contacts of men with nongonococcal urethritis) and have examined the role of only a limited number of potential pathogens. The present study of mucopurulent cervicitis was undertaken in two clinical settings to define the microbiologic etiology of cervical inflammation in consecutively referred women with suspected cervicitis and in representative samples of women from two clinical settings.

## Material and methods

**Study population.** Women attending the Seattle-King County sexually transmitted disease clinic at Har-

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borview Medical Center or the University of Washington student health center in Seattle were eligible for study. Four patient groups, two from each clinic, composed the study population: two groups of consecutive patients referred for suspected cervicitis (one at each clinic), one randomly selected group from women attending the sexually transmitted disease clinic for the first time or with a new problem, and a group of consecutive women registering for routine annual examinations at the student health clinic.

**Women referred for suspected cervicitis.** Clinicians at the sexually transmitted disease clinic and student health clinic were asked to refer women  $\geq 16$  years of age whom they believed to have cervicitis but in whom they did not suspect pelvic inflammatory disease. Women who had received antibiotics in the previous month, who were menstruating, who were pregnant, or who had an intrauterine contraceptive device were excluded. One hundred sixty-one women in the sexually transmitted disease clinic and 47 women in the student health clinic were thus referred and enrolled for suspected cervicitis.

**Representative samples of women in two clinical settings.** A random sample of 110 women attending the sexually transmitted disease clinic were selected as previously described.<sup>1</sup> At the student health clinic, 49 consecutive women registering for routine annual examinations were asked to participate in the study and all agreed.

**Patient evaluation.** A standardized history with detailed information concerning menstrual history, contraceptive method, sexual behavior, and prior history of sexually transmitted disease was obtained from all women. All women underwent a gynecologic examination by one of the investigators. The color of cervical secretions as viewed on a white cotton swab was classified as clear, white, cloudy, or yellow; yellow secretions were defined as mucopus. The severity of inflammation of the endocervical epithelium was assessed by separately scoring the degree of induced mucosal bleeding as well as erythema and edema of the zone of ectopy when observed with the unaided eye as 0 (none or normal), 1+ (mild), 2+ (moderate), or 3+ (severe) and summing the score. Bacterial vaginosis was diagnosed according to the criteria developed by Amsel et al.<sup>3</sup>

**Definition of mucopurulent cervicitis.** As previously described,<sup>1</sup> a diagnosis of mucopurulent cervicitis was established on the basis of the presence of either a yellow mucopurulent endocervical discharge or  $\geq 10$  polymorphonuclear leukocytes per 1000  $\times$  microscopic field in cervical mucus on a satisfactory Gram-stained smear. Overall, 28 smears were not evaluable because the slide contained  $>100$  squamous cells in association with inflammatory cells in cervical mucus.

**Quantification of leukocytes in endocervical secretions.** After the ectocervix was wiped clean with a large

cotton swab, endocervical mucus was collected on a white-tipped swab with care taken to avoid contamination by vaginal secretions. The swab was rolled onto a 2 cm<sup>2</sup> area of a glass microscope slide. The smear was air-dried and Gram-stained by the rapid method. Slides were then examined for the number of polymorphonuclear leukocytes per microscopic field at 1000  $\times$  magnification in five nonadjacent fields as previously described in detail.<sup>1</sup>

#### **Laboratory methods and collection of specimens.**

An unlubricated speculum was inserted in the vagina and the cervix was exposed. The lateral vaginal fornix was swabbed with a cotton-tipped applicator and vaginal pH was measured directly from this swab. A sterile paper strip (5 by 20 mm) was inserted into the endocervical canal and allowed to saturate with fluid from the cervical mucus. This was carefully done to avoid any bleeding from the endocervix during the procedure. The filter paper strip was placed in 0.2 ml of phosphate-buffered saline solution and frozen at  $-20^{\circ}$  C until assayed for antibody to *C. trachomatis*. Calcium alginate swabs (Inolex, Glenwood, Illinois) were used to obtain urethral and cervical cultures for *C. trachomatis*. These specimens were separately placed in 2 ml of sucrose-phosphate-glutamate transport medium. Subsequent successive endocervical specimens were obtained with cotton-tipped swabs for cultures for *N. gonorrhoeae*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, *Gardnerella vaginalis*, *Streptococcus agalactiae*, and herpes simplex virus. Cervicovaginal specimens were obtained for culture of *Trichomonas vaginalis* and yeast.

**Microbiologic studies.** Isolation of *C. trachomatis* was performed in two cooperating laboratories with the use of either cycloheximide-treated McCoy cells in microtiter plates or diethylaminoethyl-dextran-treated HeLa 229 cells in tubes.<sup>1</sup> *N. gonorrhoeae* was isolated by direct inoculation of the swabs on Thayer-Martin agar with incubation and identification by standard methods. Specimens were inoculated onto A7 agar medium and into broth media for isolation of *M. hominis* and *U. urealyticum*.<sup>1</sup> Broth showing evidence of growth was subcultured to A7 agar for confirmation; colonies were identified by typical morphologic features. *G. vaginalis* was isolated on human blood Tween agar medium. Colonies showing  $\beta$ -hemolysis were further identified as *G. vaginalis* as previously described.<sup>1</sup> Group B streptococci were isolated in Todd-Hewitt broth containing 5% sheep blood and 5 mg/L of gentamicin and 15 mg/L of nalidixic acid; broth was subcultured to sheep blood agar for confirmation and identification of group B streptococci on the basis of  $\beta$ -hemolytic reaction and latex agglutination with group-specific antiserum (Streptex, Wellcome Diagnostics, Research Triangle Park, North Carolina). Cervical specimens were placed in viral transport medium and inoculated into a monolayer of fibroblasts from human foreskin for



**Table I.** Associations of demographic and behavioral characteristics with mucopurulent cervicitis stratified by patient group

	STD clinic women							
	Referred				Sampled			
	MPC Neg. (n = 9)		MPC Pos. (n = 133)		MPC Neg. (n = 51)		MPC Pos. (n = 39)	
	No.	%	No.	%	No.	%	No.	%
Age (yr)	24.7 ± 4.1		23.4 ± 5.2		23.7 ± 5.7		22.3 ± 4.7	
Race white	7	78	105/132	80	33	65	28	72
Marital status single	6	67	103/130	79	35/47	75	29/38	76
Education (yr)	13.8 ± 2.1		13.2 ± 2.5		12.6 ± 1.9		12.5 ± 2.0	
Gravidity ≥1	5	56	60/132	46	24/50	48	22/38	58
Parity ≥1	1	11	41	31	12	24	7	18
Method of contraception								
Oral contraceptives	2	22	80	62	9	18	16	42
Spermicides	3	33	16	12	13	25*	4	11
Other	1	11	6	5	3	6	7	18
None	3	33	28	22	26	51	11	29
Age at first intercourse (yr)	16.6 ± 1.4		17.2 ± 2.5		16.3 ± 2.5		16.1 ± 2.1	
Median lifetime sex partners (No.)	20		8		7		10	
Past history of STD†	5/8	63	90/132	68	32	63	23/38	61

STD = Sexually transmitted disease; MPC = mucopurulent cervicitis; Neg. = negative; Pos. = positive.

\*p ≤ 0.01.

†Gonorrhea, pelvic inflammatory disease, genital warts, genital herpes, or trichomoniasis.

‡p ≤ 0.05.

isolation of herpes simplex virus.<sup>1</sup> Cervicovaginal specimens were inoculated into Diamond's medium for isolation of *T. vaginalis* and on Sabaraud's agar for isolation of yeasts.

**Serum and secretion antibody determinations.** Serum samples were tested by the microimmunofluorescence technique for IgG antibodies for all *C. trachomatis* elementary body serotypes.<sup>4</sup> Cervical secretions were similarly tested for secretory IgA antibodies to *C. trachomatis*.

**Statistical methods.** Initial comparisons of demographic, behavioral, and microbiologic characteristics of women with or without mucopurulent cervicitis were done in each of the four patient groups by standard  $\chi^2$  or *t*-tests. In order to obtain an overall assessment of the strength of the associations between possible causative factors and mucopurulent cervicitis while minimizing the statistical problems of multiple comparisons, the associations were analyzed by first stratifying patients into the four categories (referred or not referred, sexually transmitted disease or student health clinic) and then combining these results by calculating an overall odds ratio according to the Mantel-Haenszel method.<sup>5</sup> This procedure assumes that the associations are the same in all strata, and this assumption was tested for each association by use of a  $\chi^2$  test for homogeneity, which is significant if there are large disparities among the odds ratios in the four strata. The p value reported with each odds ratio corresponds to the test of the hypothesis that the odds

ratio is equal to 1 (that is, no association). These analyses were conducted with the 1981 version of the BMPD statistical program P4F.

Similar Mantel-Haenszel analyses were also conducted separately in groups of women with or without evidence of infection by *C. trachomatis* (as measured by positive cervical culture), and the results were combined into an overall statistic that adjusted for the presence of chlamydial infection.

Since the purpose of this analysis was to compare the relative strengths of the associations of several variables with mucopurulent cervicitis, the p values reported for the summary odds ratios (see Tables III, IV, and V) have not been adjusted to account for the number of associations examined.

## Results

**Incidence of mucopurulent cervicitis among referred and sampled study populations.** Thirteen of the 367 women initially enrolled (10 from the sampled sexually transmitted disease clinic population and three from the referred sexually transmitted disease clinic patient group) were menstruating and thus excluded from further evaluation because cervical findings could not be adequately assessed. The diagnosis of mucopurulent cervicitis could not be made in an additional 31 women who did not have mucopus and in whom polymorphonuclear leukocytes could not be evaluated because either the Gram-stained smear was unsatisfactory or a smear was not obtained. Of the remaining

Student health clinic women							
Referred				Sampled			
MPC Neg. (n = 8)		MPC Pos. (n = 39)		MPC Neg. (n = 29)		MPC Pos. (n = 15)	
No.	%	No.	%	No.	%	No.	%
23.6 ± 2.0		23.4 ± 3.8		23.1 ± 4.5		23.3 ± 3.8	
6	75	29/34	85	26/28	93	13/14	93
6/7	86	21/21	100	25/29	86	13/14	93
15.9 ± 1.4		16.7 ± 2.0		15.6 ± 2.0		15.0 ± 1.6	
0	0	9	23	7	24	5	33
0	0	3	8	1	3	1	7
0	0	19	50	9	31	5	33
6	75	9	24	9	31	6	40
0	0	2	5	5	17	1	7
2	25	8	21	6	21	3	20
19.8 ± 2.9		18.3 ± 3.0		18.6 ± 2.8		18.2 ± 1.9	
3		4		5		4	
0/7	10‡	11/23	48	7	24	7	47

323 patients, mucopurulent cervicitis was detected in 39 (43%) of the 90 randomly selected sexually transmitted disease clinic female patients, in 133 (94%) of the 142 sexually transmitted disease clinic patients referred for suspected cervicitis, in 15 (34%) of the 44 consecutive women seen for routine annual examination at the student health clinic, and in 39 (83%) of the 47 student health clinic patients referred for suspected cervicitis.

**Associations of demographic and behavioral characteristics with mucopurulent cervicitis in the four patient groups.** As shown in Table I, there were differences in contraceptive usage patterns among women with and without mucopurulent cervicitis. Among sexually transmitted disease clinic women, oral contraceptives were used by 96 (57%) of 168 women with mucopurulent cervicitis versus only 11 (18%) of 60 women without mucopurulent cervicitis ( $p < 0.001$ ). A similar difference in oral contraceptive usage was seen among referred student health clinic women (19 of 38 versus zero of eight,  $p < 0.01$ ) but not in the student health clinic sample. No significant differences were found in age, race, marital status, years of education, gravidity, parity, or sexual history between women with and without mucopurulent cervicitis in any of the study populations. In the referred student health clinic population, a greater proportion of women with mucopurulent cervicitis had a history of past sexually transmitted disease (11 or 48% of 23) compared with those without mucopurulent cervicitis (zero of seven,  $p < 0.05$ ).

**Associations of specific microorganisms or clinical diagnoses with mucopurulent cervicitis.** The prevalence of cervical and vaginal pathogens, clinical diag-

noses, and chlamydial antibodies among those with and without mucopurulent cervicitis stratified by patient group is shown in Table II. In separate analyses done within each group, mucopurulent cervicitis was associated with several infectious agents or clinical conditions. In the sexually transmitted disease clinic groups, the presence of mucopurulent cervicitis was most highly associated with positive cultures for *C. trachomatis*, *U. urealyticum*, or *T. vaginalis*, with the presence of serum antibodies to *C. trachomatis*, and with the clinical diagnosis of bacterial vaginosis. In the student health clinic groups, mucopurulent cervicitis was associated with the isolation of *C. trachomatis* ( $p = 0.06$ ), *T. vaginalis*, and group B streptococcus and with the clinical diagnosis of bacterial vaginosis; the isolation of yeast had a negative association with mucopurulent cervicitis.

Although isolation of herpes simplex virus was not associated with mucopurulent cervicitis, characteristic ulcerative or necrotic lesions of the cervix were observed by colposcopy in eight (67%) of 12 women from whom herpes simplex virus was isolated versus 12 (4%) of 302 women from whom herpes simplex virus was not isolated ( $p < 0.001$ ).

**Association of selected variables with mucopurulent cervicitis by the Mantel-Haenszel test.** Since many variables were associated with mucopurulent cervicitis in the separate group analyses, the comparisons of the strengths of these associations were made by determining Mantel-Haenszel summary odds ratios for each variable (Table III). Combining the odds ratios derived from all four groups was found justified by the nonsignificance of the test for homogeneity of the odds ratios from the patient groups (data not

**Table II.** Associations of cervical microorganisms, clinical diagnoses, and antichlamydial antibodies with mucopurulent cervicitis stratified by patient group

	STD clinic women							
	Referred				Sampled			
	MPC Neg. (n = 9)		MPC Pos. (n = 133)		MPC Neg. (n = 51)		MPC Pos. (n = 39)	
	No.	%	No.	%	No.	%	No.	%
<i>C. trachomatis</i>								
Cervical culture	1	11	56	42	1	2*	19	49
Serum antibody	4	44†	91/118	77	27/47	57‡	32/37	86
Cervical secretory IgA antibody	0	0	24/128	19	8/48	17	9/37	24
<i>N. gonorrhoeae</i>	0/7	0	9/132	7	7/50	14	7	18
Herpes simplex virus	0	0	7/131	5	4/50	8	1/38	3
<i>T. vaginalis</i>	0	0	12/132	9	4/50	8‡	11/38	29
<i>M. hominis</i>	3	33	55/129	43	20	40	20	51
<i>U. urealyticum</i>	4	44‡	108/130	83	37	74†	35	90
Group B streptococcus	2/8	25	21/130	16	7	14	7	18
<i>G. vaginalis</i>	5	56	95/126	75	29/47	62	29/38	76
Yeast	1/8	13	15/129	12	16/50	32	6/38	16
Bacterial vaginosis	3/8	38	44/121	36	14/50	28†	19/38	50

STD = Sexually transmitted disease; MPC = mucopurulent cervicitis; Neg. = negative; Pos. = positive.

\*p ≤ 0.001.

†p ≤ 0.05.

‡p ≤ 0.01.

**Table III.** Association of selected variables with mucopurulent cervicitis by the Mantel-Haenszel test

	Summary odds ratio	p value
<i>C. trachomatis</i>		
Cervical culture	16.39	<0.001
Serum antibody	4.24	<0.001
Cervical secretory IgA antibody	2.35	NS*
<i>N. gonorrhoeae</i>	1.66	NS
Herpes simplex virus	0.58	NS
<i>T. vaginalis</i>	3.17	0.045
<i>M. hominis</i>	1.32	NS
<i>U. urealyticum</i>	2.70	0.004
Group B streptococcus	1.14	NS
<i>G. vaginalis</i>	2.39	0.013
Yeast	0.36	0.027
Bacterial vaginosis	1.94	0.052
Oral contraceptive use	3.44	0.001
Spermicide use	0.42	0.014

\*NS = not significant; p &gt; 0.10.

shown). As shown in Table III, several variables were significantly associated with mucopurulent cervicitis, including *C. trachomatis* (odds ratio = 16.39, p < 0.001), the presence of serum antibodies to *C. trachomatis* (odds ratio = 4.24, p < 0.001), oral contraceptive use (odds ratio = 3.44, p < 0.001), isolation of *T. vaginalis* (odds ratio = 3.17, p = 0.04), isolation of *U. urealyticum* (odds ratio = 2.70, p < 0.01), isolation of *G. vaginalis* (odds ratio = 2.39, p = 0.01),

bacterial vaginosis (odds ratio = 1.94, p = 0.05), spermicide use (odds ratio = 0.42, p = 0.01, negative association), and the presence of yeast (odds ratio = 0.36, p = 0.03, negative association). Data concerning isolation of *C. trachomatis*, *N. gonorrhoeae*, and herpes simplex virus from the randomly selected sexually transmitted disease clinic women were previously used in defining the objective criteria for diagnosis of mucopurulent cervicitis and its strong relationship to *C. trachomatis*.<sup>1</sup> If these data are excluded from analysis, isolation of *C. trachomatis* remains significantly associated with mucopurulent cervicitis (odds ratio = 7.97, p < 0.01), while isolation of *N. gonorrhoeae* and herpes simplex virus still demonstrates no such association.

**Summary odds ratios for mucopurulent cervicitis after adjusting for isolation of *C. trachomatis*.** Since mucopurulent cervicitis was most strongly associated with positive *C. trachomatis* culture as measured by the largest odds ratio, we stratified the study population into those with and without cultural evidence of cervical *C. trachomatis* infection and repeated the analysis to clarify further the possible correlates of mucopurulent cervicitis. As shown in Table IV, oral contraceptive use (odds ratio = 2.55, p = 0.02), isolation of *U. urealyticum* (odds ratio = 2.39, p = 0.02), and isolation of yeast (odds ratio = 0.27, p = 0.03, negative association) were most strongly associated with mucopurulent cervicitis after adjustment for *C. trachomatis*. In addition, the presence of serum antibodies to *C. tra-*

Student health clinic women							
Referred				Sampled			
MPC Neg. (n = 8)		MPC Pos. (n = 39)		MPC Neg. (n = 29)		MPC Pos. (n = 15)	
No.	%	No.	%	No.	%	No.	%
0	0	12/38	32	1	3	2/14	14
1/4	25	20/38	53	1/15	7	2/11	18
0	0	3/38	8	1/28	4	1	7
0	0	2/38	5	0	0	0	0
0	0	0/37	0	0/26	0	0	0
1	13†	0/38	0	0/15	0	0/11	0
2	25	10/36	28	3/25	12	0/14	0
3	38	23/37	62	11/26	42	7	47
1	13†	0/38	0	0/16	0	2/10	20
3/7	43	22/37	59	5/16	31	7/10	70
1	8	2/37	5	5/16	31†	0/11	0
2	25	7	18	3	10†	5	33

*chomatis* (odds ratio = 2.20,  $p = 0.09$ ), isolation of *G. vaginalis* (odds ratio = 1.95,  $p = 0.09$ ), and bacterial vaginosis (odds ratio = 1.87,  $p = 0.09$ ) demonstrated associations with mucopurulent cervicitis after adjustment for *C. trachomatis*, although these associations were not statistically significant.

When the analysis was restricted to women with negative *C. trachomatis* cultures, odds ratios of 2.27, 2.24, 2.08, 2.03, and 1.95 were obtained for oral contraceptive use, the presence of serum antibodies to *C. trachomatis*, isolation of *G. vaginalis*, isolation of *U. urealyticum*, and the clinical diagnosis of bacterial vaginosis, respectively ( $0.05 < p < 0.1$  for each), and a significant negative association was observed for the isolation of yeast (odds ratio = 0.24,  $p < 0.02$ ). Among culture-negative women, oral contraceptive use was strongly associated with mucopurulent cervicitis in those who were seropositive (odds ratio = 6.61,  $p < 0.01$ ) but not in those who were seronegative (odds ratio = 1.5).

Among women with a positive *C. trachomatis* culture, only *U. urealyticum* ( $p < 0.01$ ) was significantly associated with mucopurulent cervicitis. Although oral contraceptive use and serum antibody to *C. trachomatis* demonstrated the highest odds ratios for mucopurulent cervicitis among those with negative cultures for *C. trachomatis*, neither was associated with mucopurulent cervicitis among those with a positive *C. trachomatis* culture.

Overall, 130 (82%) of 159 sexually transmitted disease clinic women with mucopurulent cervicitis and 31 (55%) of 56 sexually transmitted disease clinic women without mucopurulent cervicitis demonstrated cultural and/or serologic evidence of *C. trachomatis* infection. The corresponding figures among student health clinic

women were 25 (50%) of 50 and three (15%) of 20.

**Association of selected manifestations of cervical inflammation with *C. trachomatis* among women with mucopurulent cervicitis.** Since mucopurulent cervicitis was associated with several variables after adjustment for *C. trachomatis* culture, we were interested in whether specific clinical manifestations of cervicitis differed for women with and without a positive *C. trachomatis* culture. As shown in Table V, among women with mucopurulent cervicitis, several findings were more common in those who had a positive cervical culture, including cervicitis severity score  $>3$  (odds ratio = 3.45,  $p < 0.001$ ), induced mucosal bleeding (odds ratio = 2.37,  $p < 0.01$ ), and edema of the area of ectopy (odds ratio = 2.05,  $p = 0.04$ ).

### Comment

In our previous study of randomly selected sexually transmitted disease clinic women, we described objective criteria for the office diagnosis of mucopurulent cervicitis.<sup>1</sup> The presence of cervical mucus (positive swab test) and  $\geq 10$  polymorphonuclear leukocytes per 1000 $\times$  microscopic field in cervical mucus demonstrated the strongest associations with cervical infection by *C. trachomatis*, *N. gonorrhoeae*, or herpes simplex virus.

In this extended study, we analyzed the associations of multiple pathogens, clinical diagnoses, and demographic and behavioral variables with mucopurulent cervicitis. The findings we obtained among the previously reported randomly selected sexually transmitted disease clinic women were examined to see if they extended to another group of sexually transmitted disease



**Table IV.** Association of selected variables with mucopurulent cervicitis by the Mantel-Haenszel test among all patients, adjusted for *C. trachomatis*, and among patients with a negative *C. trachomatis* culture

	Among all patients, adjusted for isolation of <i>C. trachomatis</i>		Among patients with negative <i>C. trachomatis</i> culture	
	Odds ratio*	<i>p</i> Value	Odds ratio*	<i>p</i> Value
<i>C. trachomatis</i>				
Serum antibody	2.20	0.09	2.24	0.09
Cervical secretory IgA antibody	1.88	NS†	1.59	NS
<i>N. gonorrhoeae</i>	0.37	NS	0.20	NS
Herpes simplex virus	1.18	NS	1.16	NS
<i>T. vaginalis</i>	2.21	NS	1.94	NS
<i>M. hominis</i>	0.95	NS	0.93	NS
<i>U. urealyticum</i>	2.39	0.021	2.03	0.09
Group B streptococcus	0.79	NS	0.69	NS
<i>G. vaginalis</i>	1.95	0.09	2.08	0.07
Yeast	0.27	0.026	0.24	0.018
Bacterial vaginosis	1.87	0.09	1.95	0.08
Oral contraceptive use	2.55	0.021	2.27	0.06
Spermicide use	0.53	NS	0.57	NS

\*Summary odds ratio.

†NS = Not significant;  $p > 0.10$ .

clinic women (that is, those referred to us because of suspected cervicitis) and to groups of women seen in a student health clinic. We also attempted to analyze further the microbial and other correlates of mucopurulent cervicitis among women negative for *C. trachomatis*.

In separate analyses in the four patient groups, multiple infectious agents (including *C. trachomatis*, *U. urealyticum*, and *T. vaginalis*), serum antibody to *C. trachomatis*, past history of sexually transmitted disease, oral contraceptive use, and bacterial vaginosis were associated with mucopurulent cervicitis in one or more of the groups studied. On the other hand, isolation of yeast, usually *Candida albicans*, from the vagina had a negative association with mucopurulent cervicitis.

To make overall comparisons among correlates of mucopurulent cervicitis, we next combined all four groups to obtain weighted average odds ratios for each variable. This summary analysis of the combined data demonstrated four major findings. First, *C. trachomatis* detected by culture and serologic testing demonstrated the strongest association with mucopurulent cervicitis. Even after adjustment for *C. trachomatis* culture and in particular among women with negative cultures for *C. trachomatis*, the presence of serum antibodies to *C. trachomatis* remained associated with mucopurulent cervicitis (odds ratio = 2.2,  $p = 0.09$ ). This might suggest false negative cultures for *C. trachomatis* in many of the seropositive women or the persistence of abnormal cervical findings after chlamydial cervicitis among the culture-negative women who remain antibody-positive. The lack of association with secretory IgA antibodies to *C. trachomatis*, which may be a closer reflection of

current chlamydial infection, argues against the former explanation. Infections with *C. trachomatis* are characteristically chronic. Data from experimental infections suggest that the immune response to *C. trachomatis* may contribute to some of the manifestations of chronic infection.<sup>6,7</sup> The histopathologic features of chlamydial cervicitis are characterized by severe inflammatory infiltration of the stroma and prominent lymphoid follicles.<sup>8</sup> The natural history of these histopathologic changes after eradication of *C. trachomatis* has not been extensively studied. We have observed that increased vascularity and erythema of the transformation zone in the cervix persists for prolonged periods of time after treatment (Paavonen J, unpublished results). Independent association of *C. trachomatis* with mucopurulent cervicitis on serologic testing suggests persistence of these histopathologic changes in the cervix.

Second, both bacterial vaginosis and the isolation of *G. vaginalis* (a bacterial vaginosis-associated pathogen) demonstrated associations with mucopurulent cervicitis, suggesting that changes in the cervicovaginal environment might foster the development of mucopurulent cervicitis or vice versa. The redox potential at the vaginal epithelial surface is greatly reduced in bacterial vaginosis.<sup>9</sup> Oxygen consumption by polymorphonuclear leukocytes might decrease the redox potential and increase the pH of the vaginal environment to favor the growth of organisms associated with bacterial vaginosis. Mucopurulent endocervical discharge might similarly change the vaginal environment. Uniform clinical criteria for bacterial vaginosis and mucopurulent cervicitis should be used in future clinical studies

**Table V.** Association of cervical findings with results of *C. trachomatis* culture among sexually transmitted disease clinic women and student health clinic women with mucopurulent cervicitis

Cervical finding	C. trachomatis				Odds ratio*	p Value
	Positive (n = 88)		Negative (n = 133)			
	No.	%	No.	%		
Cervicitis severity score						
0-3	23	27	72	53	3.45	<0.001
4-9	63	73	63	47		
Ectopy						
Not present	10	11	21	16	1.58	NS†
Present	78	89	114	84		
Erythema of ectopy‡						
0-1	22	28	42	37	1.49	NS
2-3	56	72	72	63		
Edema of ectopy‡						
0-1	45	58	81	71	2.05	0.04
2-3	33	42	33	29		
Induced mucosal bleeding						
0-1	36	42	87	64	2.37	0.004
2-3	50	58	48	36		

\*Summary odds ratio over patient groups.

†NS = Not significant;  $p > 0.10$ .

‡Among women with ectopy.

of how the two conditions are interrelated. Does bacterial vaginosis resolve spontaneously after treatment of mucopurulent cervicitis, or should both conditions be treated concomitantly or successively? The negative association of yeast with mucopurulent cervicitis is also of interest, although it may simply be that our exclusion of women who had received antibiotics within the previous month did not exclude an effect of more remote antibiotic use, which could have promoted vulvovaginal candidiasis while eliminating mucopurulent cervicitis.

The third major finding was the strong association of oral contraceptive use with mucopurulent cervicitis. Oral contraceptive-induced hyperplasia of the endocervical epithelium might render the cervix more susceptible to many sexually transmitted disease organisms. Most prior studies have demonstrated that oral contraceptive use is associated with an increased risk of *C. trachomatis* infection.<sup>10</sup> It is not clear, however, whether the higher risk of chlamydial infection in oral contraceptive users is secondary to differences in sexual behavior or to hormonal effects of oral contraceptives. Women using oral contraceptives have a higher incidence of cervical ectopy compared with that of women with natural cycles.<sup>10</sup> Cervical ectopy may render the cervix more susceptible to chlamydial infection, or detection of *C. trachomatis* by culture might be easier among such women. Oral contraceptive use might also stimulate the growth of *C. trachomatis* in cervical epithelial cells. However, among women with negative cervical cultures for *C. trachomatis*, oral contraceptive use was correlated with mucopurulent cervicitis only

among those with serum antibody to *C. trachomatis* ( $p < 0.01$ ). This was true even after adjustment for variables reflecting sexual activity (such as age at first intercourse), suggesting that the relationship is not totally explained by increased sexual activity among women using oral contraceptives. Thus our results suggest that oral contraceptives increase the risk for mucopurulent cervicitis among women with current or past exposure to *C. trachomatis*.

The fourth finding was that *U. urealyticum* was the only organism showing a significant positive association with mucopurulent cervicitis after adjustment for results of cervical cultures for *C. trachomatis*. This association is consistent with previous studies in which *U. urealyticum* has been associated, independent of *C. trachomatis*, with nongonococcal urethritis in men.<sup>11</sup> Furthermore, several studies have associated *U. urealyticum* with puerperal infections,<sup>12</sup> low birth weight of infants,<sup>13</sup> premature labor,<sup>14</sup> subclinical endometritis,<sup>15</sup> and infertility<sup>13</sup> suggesting that *U. urealyticum* is a significant genital pathogen. In contrast, *U. urealyticum* has only occasionally been isolated from the fallopian tubes of women with salpingitis,<sup>15</sup> and previous studies have not demonstrated a causative role for *U. urealyticum* in lower genital tract infections in women. Case-control studies have demonstrated a very high incidence of *U. urealyticum* in the lower genital tract of both symptomatic and asymptomatic women.<sup>13</sup> However, a significant association of *U. urealyticum* was found in the present study with signs of a particular clinical entity, mucopurulent cervicitis. Quantitative differences in the con-

centration of *U. urealyticum* might be sought in future comparisons of women with and without mucopurulent cervicitis.

The lack of association of *N. gonorrhoeae* with mucopurulent cervicitis in sexually transmitted disease clinic patients in this study and in our previous study<sup>1</sup> was unexpected and must be interpreted with caution. It is possible that the sample of patients referred to us with suspected mucopurulent cervicitis was biased in the direction of a falsely low incidence of gonorrhea if the referring clinicians tended to start treatment for gonorrhea on the basis of cervical Gram stain results (although we tried to avoid such bias) or if women with gonorrhea tended to present to the clinic with more acute, severe clinical symptoms suggesting pelvic inflammatory disease and thus were excluded from the cervicitis study. It may also be that women with gonorrhea develop acute symptoms, leading them to seek care in other clinical settings, or that the cervicitis produced by gonorrhea is transient whereas the inflammation produced by *C. trachomatis* is longer lasting, leading to more profound or more prolonged abnormalities of the cervix. None of the referred student health clinic women with mucopurulent cervicitis in our study had gonorrhea or genital herpes. In any case, manifestations of gonococcal nonchlamydial infection of the cervix require further study.

Since our results suggest that two different types of mucopurulent cervicitis might exist, we compared chlamydial and nonchlamydial cases of mucopurulent cervicitis with respect to cervical findings on examination. Those with chlamydial cervicitis had higher cervicitis severity scores, more often had edema of the area of ectopy, and more often demonstrated induced mucosal bleeding, again suggesting that *C. trachomatis* is an invasive cervical pathogen. Subsequent studies are needed to assess our findings and to address the multifactorial etiology of cervical inflammation. Such studies should also elucidate the histopathologic characteristics of chlamydial and nonchlamydial cervicitis.

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# Magnetic resonance imaging and placenta previa

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Twenty-five women with diagnoses of placenta previa on ultrasound examination underwent magnetic resonance imaging examination. An assessment of placental position and the relationship of the lower placental edge to the internal os was made with both techniques and the results were compared. There was complete correlation of placental localization but significant differences were found in the determination of the degree of placenta previa. This occurred more often with posteriorly situated placentas. Magnetic resonance imaging directly affected management with regard to delivery in seven cases. Magnetic resonance imaging provides a technique capable of accurately assessing placental position and its relationship to the cervix, thereby leading to a reduction in hospitalization and inappropriate operations. (AM J OBSTET GYNECOL 1986;154:565-9.)

**Key words:** Magnetic resonance imaging, placenta previa, ultrasound

Placenta previa is a serious but uncommon complication of pregnancy with an incidence during the latter half of pregnancy of 0.5%, of which one fifth are of the complete or total variety.<sup>1,2</sup> Accurate localization of the placenta is important in the management of third-trimester bleeding. Ultrasound in recent years has been the investigation of choice for placental localization.<sup>3</sup> More specifically, its diagnostic accuracy with placenta previa is thought to be more than 90% in most series.<sup>4-6</sup> Identification is important because of the increased fetal and perinatal mortality with this condition.<sup>7</sup>

Magnetic resonance represents a new method of imaging that produces cross-sectional images of the body that reflect the distribution density of protons and parameters relating to their motion (the so called T<sub>1</sub> and T<sub>2</sub> relaxation times) in cellular water and lipids.<sup>8</sup> Magnetic resonance imaging has the advantage of imaging in any plane, thus avoiding the use of ionizing radiation, and when used appropriately is unassociated with any known biologic hazard. By altering the pattern of radiofrequency applied in the so-called pulse sequences, images can be produced that are weighted differently by proton density and the relaxation times T<sub>1</sub> and T<sub>2</sub>.

The potential value of magnetic resonance imaging in obstetrics is under assessment in several centers.<sup>9,10</sup> Excellent maternal soft tissue definition can be obtained; both placenta and cervix have a characteristic appearance. Therefore, the relationship of the lower edge of the placenta to the internal cervical os can be

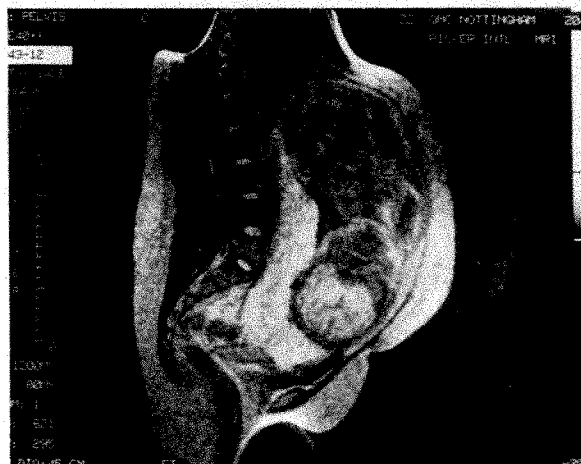


Fig. 1. Sagittal section 34 weeks; total placenta previa. Spin-echo pulse sequence TR 1200 msec TE 40 msec.

Table I. Placental localization

	Anterior	Posterior	Lateral
Ultrasound	7	16	2
Magnetic resonance imaging	7	16	2

determined. We present our initial experience with magnetic resonance imaging and the localization of the placenta in the lower segment.

## Material and methods

Twenty-five women with ultrasonic diagnoses of placenta previa in the final trimester were also examined by magnetic resonance imaging. The placental site was determined and an assessment of the relationship of the lower edge of the placenta to the internal os of the cervix was made. The placenta previa was classified as total, partial, marginal, or low-lying, the definition of

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**Fig. 2.** A and B, Sagittal section of the trilaminar cervix at 35 weeks. Spin-echo pulse sequence TR 1500 msec, TE 40 msec.

the latter being if the lower edge of the placenta is within the lower segment. The ultrasound and magnetic resonance imaging findings were compared and, where possible, the placental site and its relation to the cervix were confirmed at delivery. Examination by magnetic resonance imaging was performed within the guidelines laid down by the National Radiological Protection Board.<sup>11</sup> Informed consent was obtained in each case.

Magnetic resonance imaging was performed on a 0.15 Tesla imaging system based on a resistive magnet. A number of different spin sequences were used: a  $T_2$  weighted spin echo and a  $T_1$  weighted inversion recovery. We have found the spin-echo pulse sequences with long repetition times (TR) and long echo times (TE) to be of particular value, as both the placental tissue and the internal os of the cervix yield a high signal. The inversion recovery sequences were found to be of limited value because of poor anatomic definition and increased noise. On each patient an eight- or 16-multislice protocol was used. The sagittal views were found to be the most appropriate imaging planes as both cervix and



**Fig. 3.** A and B, Sagittal section at 34 weeks; marginal placenta previa. Spin-echo pulse sequence TR 2000 msec, TE 40 msec.

placenta are well seen. Coronal or transverse images were obtained if there was any doubt.

### Results

Magnetic resonance imaging showed itself to be equal to ultrasound in the localization of the placental site (Table I). Placental tissue has a moderately short  $T_2$  relaxation time and therefore a high-intensity signal with the use of a  $T_2$  weighted spin-echo sequence with a long TR and TE (Fig. 1). This enables the placental site to be readily identified. In contrast to the placenta, the surrounding amniotic fluid has a low signal with this spin sequence. The myometrium has an intermediate intensity signal and therefore is not always clearly discernible. The placenta in Fig. 1 is seen to be totally covering the internal os of the cervix.

It is in the precise definition of the degree of placenta previa that magnetic resonance imaging has an advantage over ultrasound (Table II). There were no false negative results with ultrasound in diagnosing the existence of a placenta previa, but in two cases the degree of placenta previa was underestimated. In 15 of 25 cases

**Table II.** Assessment of degree of placenta previa

	Total	Partial	Marginal	Low-lying	Normal
Ultrasound	5	2	13	4	1
Magnetic resonance imaging	3	4	5	8	5

**Table III.** Seven cases with management altered by magnetic resonance imaging

Case No.	Weeks' gestation	Placenta	Magnetic resonance imaging diagnosis	Ultrasound diagnosis	Management	Outcome	Weeks' gestation at outcome
1	34	Anterior	TPP	MPP	Planned cesarean section	Low-segment cesarean section	34
2	35	Posterior	MPP	TPP	Discharged home	Normal delivery	38
3	33	Posterior	Normal	MPP	Discharged home	Normal delivery	40
4	37	Posterior	LLP	MPP	Discharged home	Normal delivery	39
5	32	Posterior	LLP	MPP	Discharged home	Normal delivery	40
6	33	Posterior	MPP	LLP	Planned cesarean section	Low-segment cesarean section	38
7	28	Posterior	Normal	MPP	Discharged home	Normal delivery	41

TPP = Total placenta previa; MPP = marginal placenta previa; LLP = low-lying placenta previa.

there was a discrepancy between magnetic resonance imaging and ultrasound assessments. Subsequent ultrasound examinations at a later period of gestation confirmed the magnetic resonance imaging findings in eight other cases. The clinicians were unaware of the magnetic resonance imaging findings until a decision was to be made with regard to hospitalization and mode of delivery. In seven cases management of the placenta previa was directly altered by the magnetic resonance imaging result (Table III). In one case a cesarean section was avoided, and in another two cesarean section was performed. Five patients were discharged from the hospital as the degree of placenta previa was not as severe as that predicted by the ultrasound.

The placental site was posterior in 11 of the 15 cases where there was discrepancy between the ultrasound and magnetic resonance imaging findings. It is accepted that the ultrasound assessment of the lower placental edge may be difficult if the placenta is posterior.<sup>12</sup> In 11 of 25 patients the placental site was confirmed at delivery by cesarean section.

The cervix, when uneffaced, has a characteristic trilaminar appearance (Fig. 2, A and B). This is seen in the nonpregnant cervix but is more prominent from the early to the late stages of pregnancy. There is an outer zone of intermediate intensity surrounding a very low-intensity band, which is thought to represent stromal tissue that is high in collagen. The central zone, which has the highest intensity, is the mucus within the cervical canal. Identification of the cervix is routine with magnetic resonance imaging. In only one patient was a repeat examination required in order to visualize the cervix. The relationship of the lower edge of the placenta to the internal os therefore becomes precise.

Fig. 3, A and B, depicts sagittal scans at 34 weeks' gestation, with the use of a T<sub>2</sub> weighted pulse sequence (TR 2000 msec, TE 80 msec). The trilaminar appearance of the cervix is demonstrated well and the exact relationship of the edge of the placenta to the internal os is seen.

Although the inversion recovery sequence is unhelpful with placental localization, this sequence may facilitate the identification of retroplacental hemorrhage since hematoma gives a high signal with this sequence. Fig. 4, A is a sagittal scan of a total placenta previa at 32 weeks' gestation with the use of an inversion recovery sequence TR 2000 msec, T<sub>1</sub> 600 msec. The retroplacental high-signal area probably represents an area of hemorrhage. This patient had experienced two moderately severe bleeding episodes before examination by magnetic resonance imaging. A T<sub>2</sub> weighted spin-echo sequence is shown for comparison.

### Comment

The placental outline is clearly seen when the appropriate spin sequence is used. We have also noted that the cervix in pregnancy has a characteristic appearance with magnetic resonance imaging. Therefore, the relationship of the lower edge of the placenta to the internal os is accurately determined.

The ultrasound diagnosis of a total placenta previa is usually straightforward because of the large amount of placental tissue overlying the internal os. However, in some cases a low-lying placenta may not be distinguishable from a marginal or partial placenta previa. Acoustic shadowing from the fetal head may obscure the lower uterine segment and preclude visualization of the internal os and the lower border of the placenta.<sup>13</sup>



**Fig. 4.** A, Parasagittal section at 36 weeks; total placenta previa. Area of retroplacental hemorrhage is shown. Inversion recovery pulse sequence TR 2000 msec, T<sub>1</sub> 600 msec. B, Same section with the use of a spin-echo pulse sequence. TR 2000 msec, TE 40 msec.

Measuring the distance between the fetal skull and maternal sacrum is suggested as an indirect method of determining the presence of a posterior placenta previa but this may well be inaccurate if either the fetal skull or the cervix is not in the midline. There also may be difficulty on occasion in assessment of the anterior and lateral placenta previa.<sup>4</sup>

Overdistention of the bladder leads to compression of the placenta against both uterine walls and results in the false impression that the placenta is filling the entire lower uterine segment, which leads to a false diagnosis of placenta previa. Other sources of error are related to the skill and experience of the ultrasonographer, a large thick placenta, and cervical effacement.<sup>14</sup> Overdiagnosis by ultrasound may lead to unnecessary intervention with prolonged hospitalization and cesarean section.<sup>15</sup> Magnetic resonance imaging is not operator dependent and does not require the bladder to be filled. Magnetic resonance imaging facilitates the assessment of effacement of the cervix where it is

suspected, but the presence of placenta previa precludes vaginal examination.

Four women who were found to have marginal or partial placenta previa underwent a further examination at a 6- to 8-week interval to assess placental migration. The relationship of the lower edge of the placenta to the internal os was unchanged. The incidence of placenta previa in the second trimester has been reported as being between 5% and 45%.<sup>16</sup> Placental migration due to differential growth of the uterus is put forward as an explanation of the difference in the incidence of placenta previa in the second and third trimesters. A recent study of 250 women in the second trimester of pregnancy found no cases of placenta previa.<sup>17</sup> This, it was claimed, was due to a meticulous ultrasound examination, contradicting the previously held belief of the migrating placenta. We believe that magnetic resonance imaging may be capable of deciding this controversy once and for all. A study is now in progress.

Magnetic resonance imaging produces images capable of defining placental localization accurately and the relationship with maternal structures. Magnetic resonance imaging is a complementary examination to ultrasound in those cases where the lower limits of the placenta are not clearly visible.

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## A comparison of three techniques for ovarian reconstruction

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We compared three methods of ovarian reconstruction in 23 mature female New Zealand White rabbits. Each animal was its own control. The right ovary in each rabbit was incised and repaired by placing three 8-0 nylon sutures through and through the base of the defect (method A). The left ovary was repaired without these through-and-through sutures. In 16, hemostasis was achieved by microbipolar cautery (method B); in seven, hemostasis was achieved with three sutures placed entirely inside the defect (method B<sub>i</sub>). The cortex of each ovary was repaired identically by continuous 8-0 nylon suture. Outcome was evaluated by laparoscopy 14 days after mating. Through-and-through sutures (right ovary) caused a significant increase in adhesion formation and decrease in nidation index. When these animals were put to death 14 weeks later, the right ovaries were significantly smaller. On the left, there was no difference in any outcome variable between methods B and B<sub>i</sub>. This study clearly shows the detrimental effect of through-and-through sutures for ovarian reconstruction. (*AM J OBSTET GYNECOL* 1986;154:569-72.)

**Key words:** Ovarian reconstruction, adhesion formation, suture technique, rabbit ovary

Reconstructive ovarian surgery is generally considered to carry with it the potential for postoperative adhesion formation and mechanical infertility.<sup>1-3</sup> An improved surgical technique might decrease adhesion formation and result in a higher intrauterine pregnancy rate. Two different techniques for reconstructing the ovaries following wedge resection or ovarian cystectomy have been described. The first technique, described by Kistner and Patton,<sup>4</sup> by Tovell,<sup>5</sup> and by Rock and Jones,<sup>6</sup> involves a two-layer closure. The first layer consists of interrupted or continuous mattress sutures through and through the base of the ovarian defect, just above the hilus, closing dead space and securing hemostasis. This technique is commonly referred to as the Buxton stitch.<sup>7</sup> The second layer approximates ovarian cortex with either interrupted or continuous sutures.

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A second technique, described by Boeckx et al.<sup>8</sup> and by Gomel,<sup>9</sup> also uses a two-layer closure. The ovarian defect is closed by interrupted sutures placed entirely within the ovary. The ovarian cortex is closed in a second layer of continuous suture.

A third alternative would be to avoid the first layer of sutures altogether and instead achieve hemostasis in the ovarian defect with cautery. The ovarian cortex could again be closed in a layer of continuous suture.

The purpose of this study was to compare these three methods of ovarian reconstruction with regard to their potential for postoperative adhesion formation and their effect on reproductive performance in the rabbit.

### Material and methods

Twenty-three sexually mature female New Zealand white rabbits, aged 15 to 18 months and weighing between 2500 and 3300 gm were used. General anesthesia was induced with intramuscular ketamine hydrochloride, 20 mg/kg of body weight, and continued with a mixture of oxygen/nitrous oxide and Fluothane by face mask. The ovaries were exposed on each side by separate flank incisions. Each ovary was longitudinally bisected from one pole to the other to a depth just above the hilus. The right ovary in all 23 rabbits was repaired



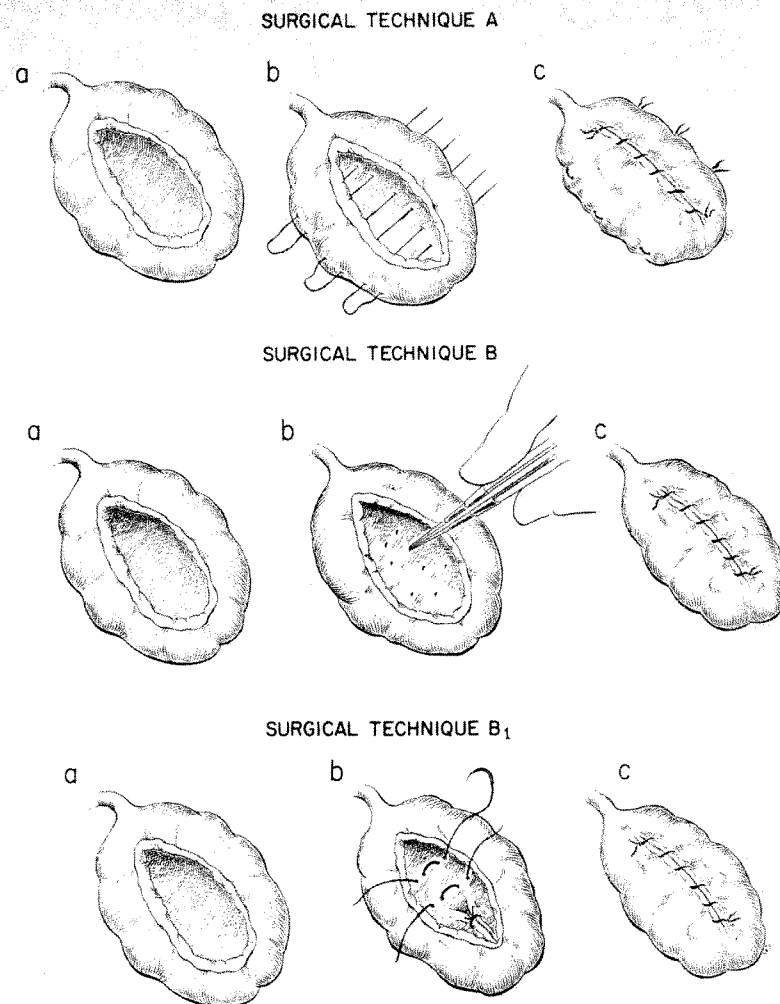


Fig. 1. Three surgical techniques for ovarian reconstruction.

by placing three interrupted mattress stitches through and through the ovary at the base of the defect. The ovarian cortex was approximated with a continuous suture (surgical technique A). In 16 rabbits hemostasis was achieved within the defect of the left ovary by meticulous microbipolar coagulation, and the cortex was carefully approximated with use of a continuous suture (surgical technique B). The left ovary of the remaining seven rabbits was repaired by placing three interrupted sutures entirely inside the ovary to control bleeding and to approximate the defect. The ovarian cortex was again approximated with use of a running suture (surgical technique B<sub>1</sub>) (Fig. 1). No mattress sutures were used on any of the left ovaries.

The Carl Zeiss operating microscope was used for all operations. Microsurgical principles, including continuous irrigation, gentle tissue handling, use of microsurgical instruments, and meticulous hemostasis by means of microbipolar coagulation were routinely ob-

served for all operations. An 8-0 monofilament nylon suture mounted on a 6 mm taper needle was used exclusively.

Five weeks following surgery the rabbits were mated with a buck of proven fertility. Eleven to 14 days after mating a second-look laparotomy was performed. The number of corpora lutea on each ovary and the number of embryos in each uterine horn were counted and the nidation index calculated. Adhesion formation on each side was evaluated. The following system was used for adhesion grading: grade 0 = no adhesions; grade 1 = filmy avascular adhesions at the operation site and between ovary and fimbria; grade 2 = thin vascular adhesions between the ovary and fimbria and surrounding organs; grade 3 = dense, opaque vascular adhesions encapsulating the ovary and surrounding organs.

The pregnancies were allowed to continue to term. Twelve to 14 weeks after surgery each rabbit was ex-

**Table I.** Adhesion grades for group B compared to group B<sub>1</sub> (left ovary)

Group	No. of rabbits	No. of rabbits by adhesion grade				Median adhesion grade
		0	I	II	III	
B*	16	8	7	1	0	0
B <sub>1</sub>	7	4	2	1	0	0

\*p = 0.738 (compared to group B<sub>1</sub>).

**Table II.** Mean nidation index for group B compared to group B<sub>1</sub> (left ovary)

Group	No. of rabbits	Nidation index (mean ± SE)
B*	16	0.980 ± 0.014
B <sub>1</sub>	7	0.905 ± 0.050

\*p = 0.066 (compared to B<sub>1</sub>).

plored through a midline abdominal incision, and the ovaries were removed for weighing and histologic examination in the nonpregnant state.

To evaluate these three reconstructive techniques, the following parameters were compared: (1) grade of adhesion formation on each side, (2) pregnancy rate (number of rabbits conceiving on each side), and (3) nidation index. Nidation index was calculated as the number of embryos divided by the number of corpora lutea on each side. Each animal served as its own control.

## Results

None of the 23 rabbits operated on died following the operation. A total of 46 ovaries were evaluated.

At second-look laparotomy the grades of adhesions present on the left side in groups B and B<sub>1</sub> were compared. Eight (50%) of the 16 ovaries subjected to surgical technique B and three (43%) of the seven ovaries operated on by surgical technique B<sub>1</sub> had periadnexal adhesions. Only one adnexa in each of these groups (B and B<sub>1</sub>) had grade II adhesions. None of them had grade III adhesions. There was no significant difference in degree of adhesions stimulated by the two techniques (Fisher's randomization test) (Table I). The numbers of embryos and corpora lutea in the left ovaries of groups B and B<sub>1</sub> were compared. No significant differences were found. Consequently the nidation index of the two groups did not differ (Table II). There was also no difference in the weight of the ovaries of groups B and B<sub>1</sub> (Table III). Since the two groups did not differ with regard to any of these variables, the two groups were not considered to be different and were therefore combined. The left and right sides of all 23 rabbits were then compared.

**Table III.** Mean ovarian weight for group B compared to group B<sub>1</sub> (left ovary)

Group	No. of rabbits	Weight (gm)
B*	16	0.252 ± 0.019
B <sub>1</sub>	7	0.263 ± 0.014

\*p = 0.722 (compared to B<sub>1</sub>).

Of the 23 rabbits, 17 had a higher grade of adhesions on the right side, one had a higher grade of adhesions on the left side, and five had the same grade of adhesions on both sides. A sign test on these data revealed significantly greater adhesion formation on the right side (Table IV).

A paired *t* test revealed no significant differences in the left and right ovaries in the number of corpora lutea (*p* = 0.790). There was, however, a significant difference in the number of embryos in the left and right uteri (*p* < 0.001) (Table V), and consequently a significant difference in the nidation index in the two sides (Table VI). The mean weight of the right ovaries was significantly less than the weight of the left ovaries (Table VII).

## Comment

Although ovarian cystectomy is one of the most frequent gynecologic operations performed in young women, there are still significant differences in the techniques employed for ovarian reconstruction. These techniques may vary in their potential for postoperative adhesion formation and mechanical infertility. In this study, with use of the rabbit ovary as a model, the superiority of techniques B and B<sub>1</sub> over technique A is clearly demonstrated. There were significantly fewer adnexal adhesions when no mattress sutures were placed (Table IV). External placement of suture material in the fashion of a Buxton stitch encourages a foreign body reaction. In addition, mattress sutures may cause vascular compression and promote ischemia and additional adhesion formation.

The nidation index, which reflects the efficacy of each tuboovarian-uterine unit, clearly demonstrates the detrimental effect of mattress sutures in reconstructive

**Table IV.** Adhesion grade with left compared to right for all 23 rabbits

Side	No. of rabbits	No. of rabbits by adhesion grade				Median adhesion grade
		0	I	II	III	
Right*	23	3	2	8	10	II
Left	23	12	9	2	0	0

\* $p < 0.0001$  (compared to left).**Table V.** Mean number of corpora lutea and embryos for left compared to right side

Side	No. of rabbits	Corpora lutea (n)	Embryos (n)
Right	23	4.609 $\pm$ 0.319*	2.783 $\pm$ 0.377†
Left	23	4.478 $\pm$ 0.387	4.261 $\pm$ 0.368

\* $p = 0.790$  (compared to left).† $p < 0.001$  (compared to left).**Table VI.** Mean nidation index for left compared to right

Side	No. of rabbits	Nidation index
Right*	23	0.583 $\pm$ 0.055
Left	23	0.957 $\pm$ 0.019

\* $p < 0.001$  (compared to left).

ovarian surgery. The nidation index was significantly higher in both techniques used on the left side when mattress sutures were avoided (Table VI). The number of corpora lutea on each side did not differ, but there were significantly fewer embryos on the right side, a direct result on the amount of adhesions encountered there. These adhesions are probably responsible for a derangement in ovum pick-up and consequently a lower nidation index.

The mattress sutures did not compromise the endocrine function of the ovary, since the number of corpora lutea were equal on both sides. They did, however, cause the ovary to become smaller on the left side (Table VI). Perhaps mattress sutures placed at the base of the ovarian defect compromise ovarian blood supply, although histologic studies revealed no increase in fibrosis. In contrast to mattress sutures, stitches placed inside the ovary in technique B<sub>1</sub> did not result in more adhesions, smaller ovaries, or fewer corpora lutea.

In conclusion, this study clearly shows the detrimen-

**Table VII.** Mean ovarian weight of left compared to right ovaries for all 23 rabbits

Side	No. of rabbits	Weight (gm)
Right*	23	0.202 $\pm$ 0.010
Left	23	0.255 $\pm$ 0.014

\* $p < 0.001$  (compared to left).

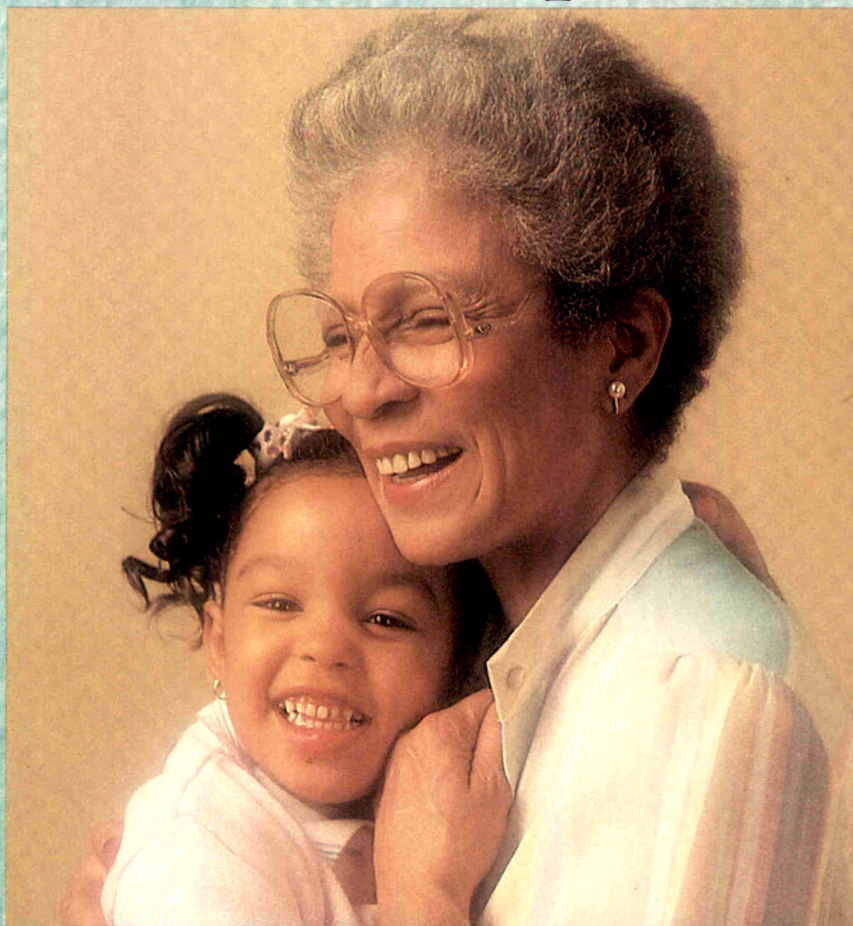
tal effect of through-and-through mattress sutures for ovarian reconstruction, in terms of adhesion formation, nidation index, and ovarian weight. Mattress sutures not only encourage a foreign body reaction but may also compromise vascular flow and promote ischemia, furthering adhesion formation. Our results, although derived from the rabbit ovary model, have important implications for reconstruction of the human ovary. We recommend complete avoidance of mattress sutures.

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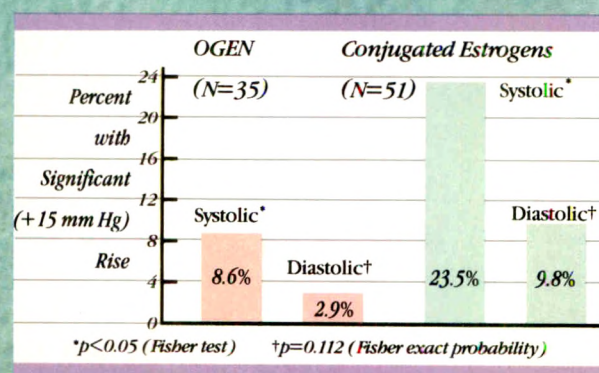
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# Her estrogen therapy shouldn't affect her blood pressure



**Percent of patients showing BP change of 15 mm Hg or greater after 7.4 months to 7.9 months therapy with either conjugated estrogens or OGEN® (estropipate)**



*Adapted from Wren et al, 1981!*

OGEN dosages ranged from 0.625 to 2.5 daily, 24 out of 30 days. Conjugated estrogen dosages ranged from 0.3 to 2.5, 24 out of 30 days. The majority of those experiencing a change, like the majority of the total sample, were also taking a progestogen, levonorgestrel 0.03 mg, from the 15th to the 24th day.

Because the risk of hypertension increases with age, it is appropriate to examine the effects of estrogen replacement on blood pressure.

"More women receiving...[conjugated estrogens] had a clinically significant rise in blood pressure than those receiving Ogen..."<sup>1</sup>

**OGEN®**  
(estropipate)

*Only estrone. Identical to the body's own.*



# Scored tablets make precise dosing easy



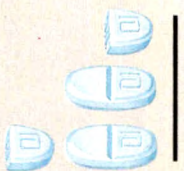
## OGEN .625

(estropipate 0.75 mg, calculated as  
sodium estrone sulfate 0.625 mg)



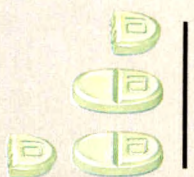
## OGEN 1.25

(estropipate 1.5 mg, calculated as  
sodium estrone sulfate 1.25 mg)



## OGEN 2.5

(estropipate 3 mg, calculated as  
sodium estrone sulfate 2.5 mg)



## OGEN 5

(estropipate 6 mg, calculated as  
sodium estrone sulfate 5 mg)

■ OGEN tablets are scored so you can adjust dosage without writing a new prescription. It's another reason more physicians are prescribing OGEN.

See adjacent page for brief summary of prescribing information.

**Reference:**

1. Wren BG. Routledge DA: Blood pressure changes: Oestrogens in climacteric women. *Med J Aust* 2:528-531, 1981.

OGEN  
(estropipate)

*Only estrone. Identical to the body's own*





# **OGEN®** ESTROPIRATE TABLETS, USP Tablets

## **WARNING:**

### **1. ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA.**

Three independent case control studies have shown an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for prolonged periods. This risk was independent of the other known risk factors for endometrial cancer. These studies are further supported by the finding that incidence rates of endometrial cancer have increased sharply since 1969 in eight different areas of the United States with population-based cancer reporting systems, an increase which may be related to the rapidly expanding use of estrogens during the last decade.

The three case control studies reported that the risk of endometrial cancer in estrogen users was about 4.5 to 13.9 times greater than in nonusers. The risk appears to depend on both duration of treatment and on estrogen dose. In view of these findings, when estrogens are used for the treatment of menopausal symptoms, the lowest dose that will control symptoms should be utilized and medication should be discontinued as soon as possible. When prolonged treatment is medically indicated, the patient should be reassessed on at least a semiannual basis to determine the need for continued therapy. Although the evidence must be considered preliminary, one study suggests that cyclic administration of low doses of estrogen may carry less risk than continuous administration; it therefore appears prudent to utilize such a regimen.

Close clinical surveillance of all women taking estrogens is important. In all cases of undiagnosed persistent or recurring abnormal vaginal bleeding, adequate diagnostic measures should be undertaken to rule out malignancy.

There is no evidence at present that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.

### **2. OGEN SHOULD NOT BE USED DURING PREGNANCY.**

According to some investigators, the use of female sex hormones, both estrogens and progestogens, during early pregnancy may seriously damage the offspring. Studies have reported that females exposed in utero to diethylstilbestrol, a non-steroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. In one of these studies, this risk was estimated as not greater than 4 per 1000 exposures. Furthermore, there are reports that a high percentage of such exposed women (from 30 to 90 percent) have been found to have vaginal adenosis, epithelial changes of the vagina and cervix. Although these reported changes are histologically benign, the investigators have not determined whether they are precursors of adenocarcinoma.

Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies in the offspring, including heart defects and limb reduction defects. One case control study estimated a 4.7 fold increased risk of limb reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 per 1000.

In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. OGEN has not been studied for these uses, and therefore should not be used during pregnancy. There is no evidence from well controlled studies that progestogens are effective for these uses.

If OGEN (estropipate tablets) is used during pregnancy, or if the patient becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus, and the question of continuation of the pregnancy should be addressed.

## **INDICATIONS AND USAGE**

The cyclic administration (See "DOSAGE AND ADMINISTRATION" section) of OGEN (estropipate tablets) is indicated for the treatment of estrogen deficiency associated with:

1. Moderate to severe vasomotor symptoms of menopause. (There is no evidence that estrogens are effective for nervous symptoms or depression which might occur during menopause, and they should not be used to treat these conditions.)
2. Atrophic vaginitis.
3. Kraurosis vulvae.
4. Female hypogonadism.
5. Female castration.
6. Primary ovarian failure.

**OGEN (ESTROPIRATE TABLETS) HAS NOT BEEN TESTED FOR EFFICACY FOR ANY PURPOSE DURING PREGNANCY. SINCE ITS EFFECT UPON THE FETUS IS UNKNOWN, IT CANNOT BE RECOMMENDED FOR ANY CONDITION DURING PREGNANCY (SEE BOXED WARNING).**

## **CONTRAINDICATIONS**

OGEN should not be used in women with any of the following conditions.

1. Known or suspected cancer of the breast.
2. Known or suspected estrogen-dependent neoplasia.
3. OGEN may cause fetal harm when administered to a pregnant woman. OGEN is contraindicated in women who are or may become pregnant (See Boxed Warning).
4. Undiagnosed abnormal genital bleeding.
5. Active thrombophlebitis or thromboembolic disorders.
6. A past history of thrombophlebitis, thrombosis, or thromboembolic disorders associated with previous estrogen use.

## **WARNINGS**

**1. Induction of malignant neoplasms.** Long-term continuous administration of natural and synthetic estrogens in certain animal species has been reported by some investigators to increase the frequency of carcinomas of the breast, cervix, vagina, and liver. There is now evidence that estrogens increase the risk of carcinoma of the endometrium in humans. (See Boxed Warning).

At the present time there is no conclusive evidence that estrogens given to postmenopausal women increase the risk of cancer of the breast. There are, however, a few retrospective studies which suggest a small but statistically significant increase in the risk factor for breast cancer among these women. Therefore, caution should be exercised when administering estrogens to women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease, or abnormal mammograms. Careful breast examinations should be performed periodically.

**2. Gall bladder disease.** A recent study has reported a 2 to 3-fold increase in the risk of surgically confirmed gall bladder disease in women receiving postmenopausal estrogens, similar to the 2-fold increase previously noted in users of oral contraceptives. In the case of oral contraceptives, the increased risk appeared after two years of use.

**3. Effects similar to those caused by estrogen-progestogen oral contraceptives.** There are several serious adverse effects of oral contraceptives, most of which have not, up to now, been documented as consequences of postmenopausal estrogen therapy. This may reflect the comparatively low doses of estrogen used in postmenopausal women. It would

be expected that the larger doses of estrogen used to treat postpartum breast engorgement would be more likely to result in these adverse effects, and, in fact, it has been shown that there is an increased risk of thrombosis in women receiving estrogens for postpartum breast engorgement.

**a. Thromboembolic disease.** It is now well established that users of oral contraceptives have an increased risk of various thromboembolic and thrombotic vascular diseases, such as thrombophlebitis, pulmonary embolism, stroke, and myocardial infarction. Cases of retinal thrombosis, mesenteric thrombosis, and optic neuritis have been reported in oral contraceptive users. There is evidence that the risk of several of these adverse reactions is related to the dose of the drug. An increased risk of post-surgery thromboembolic complications has also been reported in users of oral contraceptives. If feasible, estrogen should be discontinued at least 4 weeks before surgery of the type associated with an increased risk of thromboembolism; it should also be discontinued during periods of prolonged immobilization.

While an increased rate of thromboembolic and thrombotic disease in postmenopausal users of estrogens has not been found it does not rule out the possibility that such an increase may be present or that subgroups of women who have underlying risk factors or who are receiving relatively large doses of estrogens may have increased risk. Therefore estrogens should not be used in persons with active thrombophlebitis or thromboembolic disorders, and they should not be used in persons with a history of such disorders in association with estrogen use. They should be used with caution in patients with cerebral vascular or coronary artery disease and only for those in whom estrogens are clearly needed.

Large doses of estrogen (5 mg conjugated estrogens per day), comparable to those used to treat cancer of the prostate and breast, have been shown in a large prospective clinical trial in men to increase the risk of nonfatal myocardial infarction, pulmonary embolism and thrombophlebitis. When estrogen doses of this size are used, any of the thromboembolic and thrombotic adverse effects associated with oral contraceptive use should be considered a clear risk.

**b. Hepatic adenoma.** Benign hepatic adenomas appear to be associated with the use of oral contraceptives. Although benign, and rare, these may rupture and cause death through intraabdominal hemorrhage. Such lesions have not yet been reported in association with other estrogen or progestogen preparations but should be considered in estrogen users having abdominal pain and tenderness, abdominal mass, or hypovolemic shock. Hepatocellular carcinoma has also been reported in women taking estrogen-containing oral contraceptives. The relationship of this malignancy to these drugs is not known at this time.

**c. Elevated blood pressure.** Increased blood pressure is not uncommon in women using oral contraceptives. There is now a report that this may occur with use of estrogens in the menopause and blood pressure should be monitored with estrogen use, especially if high doses are used.

**d. Glucose tolerance.** A worsening of glucose tolerance has been observed in a significant percentage of patients on estrogen-containing oral contraceptives. For this reason, diabetic patients should be carefully observed while receiving estrogen.

**4. Hypercalcemia.** Administration of estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases. If this occurs, the drug should be stopped and appropriate measures taken to reduce the serum calcium level.

## **PRECAUTIONS**

### **A. General Precautions.**

1. A complete medical and family history should be taken prior to the initiation of any estrogen therapy. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen, and pelvic organs, and should include a Papanicolaou smear. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination being performed.

2. Fluid retention — Estrogens may cause some degree of fluid retention. Therefore, patients with conditions such as epilepsy, migraine, and cardiac or renal dysfunction, which might be influenced by this factor, require careful observation.

3. Certain patients may develop undesirable manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding, mastodynia, etc.

4. Oral contraceptives appear to be associated with an increased incidence of venous thromboembolism. Although it is not clear whether this is due to the estrogenic or progestogenic component of the contraceptive, patients with a history of depression should be carefully observed.

5. Preexisting uterine leiomyomata may increase in size during estrogen use.

6. The pathologist should be advised of the patient's use of estrogen therapy when relevant specimens are submitted.

7. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving estrogen-containing oral contraceptive therapy. If jaundice develops in any patient receiving estrogen, the medication should be discontinued while the cause is investigated.

8. Estrogens may be poorly metabolized in patients with impaired liver function and they should be administered with caution in such patients.

9. Because estrogens influence the metabolism of calcium and phosphorus, they should be used with caution in patients with metabolic bone diseases that are associated with hypercalcemia or in patients with renal insufficiency.

B. Information for the Patient. See text of Patient Package Insert which appears after PHYSICIAN REFERENCES.

C. Drug Interactions. The concomitant use of any drugs which can induce hepatic microsomal enzymes with estrogens may produce estrogen levels which are lower than would be expected from the dose of estrogen administered.

The use of broad spectrum antibiotics which profoundly effect intestinal flora may influence the absorption of steroidal compounds including the estrogens.

Diabetics receiving insulin may have increased insulin requirements when receiving estrogens.

Laboratory Test Interference. Certain endocrine and liver function tests may be affected by estrogen-containing oral contraceptives. The following similar changes may be expected with larger doses of estrogen:

- a. Increased sulfobromophthalein retention.
- b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin III; increased norepinephrine-induced platelet aggregability.
- c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by T<sub>4</sub> T<sub>3</sub> column, or T<sub>4</sub> by radioimmunoassay. Free T<sub>3</sub> resin uptake is decreased, reflecting the elevated TBG; free T<sub>4</sub> concentration is unaltered.
- d. Abnormal glucose tolerance test results.
- e. Decreased pregnandiol excretion.
- f. Reduced response to metyrapone test.
- g. Reduced serum folate concentration.
- h. Increased serum triglyceride and phospholipid concentration.

D. Carcinogenesis. Studies have shown an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for prolonged periods (see Boxed Warning). At the present time there is no conclusive evidence that estrogens given to postmenopausal women increase the risk of cancer of the breast. There are, however, a few retrospective studies which suggest a small but statistically significant increase in the risk factor for breast cancer among these women. (See "WARNINGS" section.)

E. Pregnancy, Pregnancy Category X. See "CONTRAINDICATIONS" section and Boxed Warning.

F. Nursing Mothers. Estrogens have been reported to be excreted in human breast milk. Caution should be exercised when OGEN is administered to a nursing woman.

G. Pediatric Use. Because of the effects of estrogens on epiphyseal closure, they should be used judiciously in young patients in whom bone growth is not complete.

## **ADVERSE REACTIONS**

(See Warnings regarding reports of possible induction of neoplasia, unknown effects upon the fetus, increased incidence of gall bladder disease, and adverse effects similar to those of oral contraceptives, including thromboembolism.) The following additional adverse reactions in decreasing order of severity within each category have been reported with estrogenic therapy, including oral contraceptives:

1. **Genitourinary system.**  
Increase in size of uterine fibromyoma.  
Vaginal candidiasis.  
Cystitis-like syndrome.  
Dysmenorrhea.  
Amenorrhea during and after treatment.  
Change in cervical eversion and in degree of cervical secretion.  
Breakthrough bleeding, spotting, change in menstrual flow.  
Premenstrual-like syndrome.
2. **Breast.**  
Tenderness, enlargement, secretion.
3. **Gastrointestinal.**  
Cholestatic jaundice.  
Nausea, vomiting.  
Abdominal cramps, bloating.
4. **Skin.**  
Hemorrhagic eruption.  
Erythema nodosum.  
Erythema multiforme.  
Hirsutism.  
Chloasma or melasma which may persist when drug is discontinued.  
Loss of scalp hair.
5. **Eyes.**  
Steepening of corneal curvature.  
Intolerance to contact lenses.
6. **CNS.**  
Chorea.  
Mental depression.  
Migraine, dizziness, headache.
7. **Miscellaneous.**  
Aggravation of porphyria.  
Edema.  
Reduced carbohydrate tolerance.  
Increase or decrease in weight.  
Changes in libido.

## **OVERDOSAGE**

Numerous reports of ingestion of large doses of estrogen-containing oral contraceptives by young children indicate that serious ill effects do not occur. Overdosage of estrogen may cause nausea and withdrawal bleeding may occur in females.

## **DOSAGE AND ADMINISTRATION**

### **1. Given cyclically for short-term use:**

For treatment of moderate to severe vasomotor symptoms, atrophic vaginitis, or kraurosis vulvae associated with the menopause.

The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible.

Administration should be cyclic (e.g., 3 weeks on and 1 week off). Attempts to discontinue or taper medication should be made at 3 to 6 month intervals.

Usual dosage ranges:

**Vasomotor symptoms** — One OGEN .625 Tablet to one OGEN 5 Tablet per day. The lowest dose that will control symptoms should be chosen. If the patient has not menstruated within the last two months or more, cyclic administration is started arbitrarily. If the patient is menstruating, cyclic administration is started on day 5 of bleeding.

**Atrophic vaginitis and kraurosis vulvae** — One OGEN .625 Tablet to one OGEN 5 Tablet daily, depending upon the tissue response of the individual patient. The lowest dose that will control symptoms should be chosen. Administer cyclically.

### **2. Given cyclically:**

Female hypogonadism; female castration; primary ovarian failure.

Usual dosage ranges:

**Female hypogonadism** — A daily dose of one OGEN 1.25 Tablet to three OGEN 2.5 Tablets may be given for the first three weeks of a theoretical cycle, followed by a rest period of eight to ten days. The lowest dose that will control symptoms should be chosen. If bleeding does not occur by the end of this period, the same dosage schedule is repeated. The number of courses of estrogen therapy necessary to produce bleeding may vary depending on the responsiveness of the endometrium. If satisfactory withdrawal bleeding does not occur, an oral progestogen may be given in addition to estrogen during the third week of the cycle.

**Female castration and primary ovarian failure** — A daily dose of one OGEN 1.25 Tablet to three OGEN 2.5 Tablets may be given for the first three weeks of a theoretical cycle, followed by a rest period of eight to ten days. Adjust dosage upward or downward according to severity of symptoms and response of the patient. For maintenance, adjust dosage to lowest level that will provide effective control.

Treated patients with an intact uterus should be monitored closely for signs of endometrial cancer and appropriate diagnostic measures should be taken to rule out malignancy in the event of persistent or recurring abnormal vaginal bleeding.

## **HOW SUPPLIED**

OGEN (estropipate tablets, USP) is supplied as OGEN .625 (0.75 mg estropipate), yellow tablets, NDC 0074-3943-04; OGEN 1.25 (1.5 mg estropipate), peach-colored tablets, NDC 0074-3946-04; OGEN 2.5 (3 mg estropipate), blue tablets, NDC 0074-3951-04; and OGEN 5 (6 mg estropipate), light green tablets, NDC 0074-3958-13. Tablets of all four dosage levels are standardized to provide uniform estrone activity and are grooved (Divide-Tab®) to provide dosage flexibility. All tablet sizes of OGEN are available in bottles of 100.

5043718/R1



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### CHIEF OF PERINATOLOGY

The Bryn Mawr Hospital, a 406-bed community hospital affiliated with Thomas Jefferson University, is seeking a Board certified or Board eligible Perinatologist to develop a regional perinatology program.

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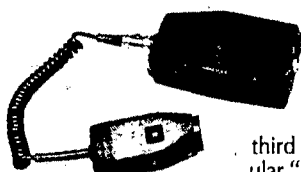
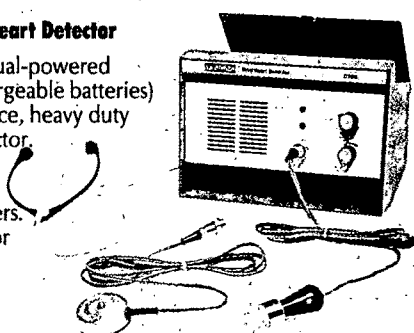


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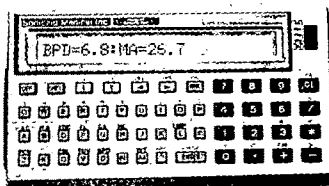


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# A double-blind placebo-controlled trial of progesterone vaginal suppositories in the treatment of premenstrual syndrome

Sarah Maddocks, M.A.,\* Philip Hahn, M.Sc., Frederick Moller, Ph.D., and Robert L. Reid, M.D.\*\*

Kingston, Ontario, Canada

Rigorous criteria were used to select women with severe premenstrual syndrome for inclusion in an 8-month double-blind placebo-controlled clinical trial of progesterone vaginal suppositories. Following a control month without treatment, progesterone (200 mg in polyethylene glycol base) or placebo was self-administered twice daily by vaginal suppository for a minimum of 12 days before the onset of menstruation for 3 months. Crossover to the opposite medication for a further 3 months was followed by a final control cycle without treatment in month 8. Physician contact was minimized throughout the study to avoid any possible positive effects of psychological support which may have confounded past investigations. Detailed self-report questionnaires were completed every 3 days for the duration of the study. Although the attrition rate was high, 20 women completed the trial and their records are analyzed here. The results of this trial indicate that the response to vaginal progesterone in these dosages is, at best, marginal and not significantly different from response with placebo use. (AM J OBSTET GYNECOL 1986;154:573-81.)

**Key words:** Premenstrual syndrome, treatment, progesterone vaginal suppositories, double-blind study

Premenstrual syndrome may be defined as the cyclic recurrence in the luteal phase of the menstrual cycle of a combination of distressing physical, psychological, and/or behavioral changes of sufficient severity to result in deterioration of interpersonal relationships and/or interference with normal activities.<sup>1</sup> Although a causal link between premenstrual syndrome and the cyclic function of the hypothalamic-pituitary-ovarian axis seems certain, the precise pathophysiology of this disorder remains obscure. The many conflicting theories, each with its own therapeutic possibilities, have both confused and frustrated clinicians faced with the task of treating afflicted women. The realization that women suffering from premenstrual syndrome are particularly vulnerable to the promotion of unproven and potentially costly treatments offered by specialized premenstrual syndrome clinics has led to renewed calls for more rigorous validation procedures.<sup>2</sup> Progesterone therapy remains one of the most controversial treatments for premenstrual syndrome because it con-

tinues to be promoted despite the fact that conclusive evidence about its efficacy has failed to materialize since it was first employed in 1938.<sup>3</sup>

We report here the results of a long-term double-blind placebo-controlled trial of progesterone therapy in the treatment of severe premenstrual syndrome.

## Methods

**Subjects.** Between January 1 and June 30, 1983, 79 women were seen in the Reproductive Endocrinology Clinic in Kingston General Hospital with a primary referring diagnosis of premenstrual syndrome. Regularly menstruating patients, aged 18 to 45, who were using a nonhormonal method of contraception, were carefully screened to determine their suitability for a clinical trial of progesterone vaginal suppositories, which had been approved by the Queen's University Ethics Committee. Those with past or present psychiatric diagnoses, those with coexisting medical or gynecologic disorders (endometriosis, abnormal vaginal bleeding, etc.), and those on other medications were excluded at the start.

Patients providing a retrospective account of premenstrual physical, psychological, or behavioral change of sufficient severity to disrupt their interpersonal relationships and activities of daily living were assessed in detail by means of the Self-Rating and the Observer-Rating Scales for Premenstrual Tension Syndrome and by the Research Diagnostic Criteria of Steiner et al.<sup>4</sup> Only individuals fulfilling the Research Diagnostic Criteria and scoring consistently higher than 18 on both

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*\*Ph.D. Candidate, Clinical Psychology, supported by Franklin Bracken Fellowship, Queen's University, Kingston, Ontario.*

*\*\*Supported by the Medical Research Council of Canada Grants DG289 and DG290.*

**Table I.** Demographic characteristics of the 20 women with records subjected to data analysis

Age	No.	Parity	No.	Marital status	No.
18-20	1	0	3	Single	3
21-25	2	1	6	Married	12
26-30	4	2	8	Separated/divorced	5
31-35	4	3	1		
36-40	7	4	1		
41-45	2				

their self rating and on two independent (S. M. and R. L. R.) observer ratings were asked to consent to participation in the clinical trial.

Of the 79 women originally referred with premenstrual syndrome, 27 were excluded from consideration for the trial based on information derived from the initial interview. Primary reasons for these exclusions included age restrictions (1), failure to use nonsteroidal contraception (5), other psychiatric diagnoses and/or medications (6), other medical/gynecologic diagnoses and/or medications (13), irregular menstruation (1), and insufficient severity (1).

Forty-eight of the remaining 52 women consented to participate in a double-blind placebo-controlled trial of progesterone vaginal suppositories.

**Study design.** The study design incorporated a minimum of 1 month of baseline documentation followed by 3 months of treatment (or placebo) with crossover to the opposite therapy for a further 3 months and 1 final month of posttreatment evaluation. Patients were randomly assigned to treatment schedules by Kingston General Hospital Pharmacy, thereby adhering to the double-blind design of the trial.

Subjects visited the hospital once monthly to return completed questionnaires and to pick up new questionnaires and appropriate medication for the following month. To avoid possible modifying effects of counseling, group therapy, or physician contact, patients in this trial had no direct contact with those supervising the trial at these or other times unless some specific complications arose.

To ensure the diagnosis of premenstrual syndrome and to accurately document response to therapy, rigorous concurrent documentation was obtained by means of a battery of self-report questionnaires every 3 days throughout the entire study.

Subjects were encouraged to complete these forms concurrently at the correct times by the requirement that they leave a brief message on each occasion on a telephone recording device. The psychometric instruments used included the following:

*Measures of depression, irritability, and anxiety*

1. The Beck Depression Inventory<sup>5</sup>—a 21-item self-report questionnaire (each rated 0-3) designed to assess a subject's current depth of depression (scores: 0-9, not

depressed; 10-15, mild; 16-23, moderate; 24-63, severe depression).

2. Buss Durkee Irritability Scale—an 11-item subscale of a Guilt Hostility Inventory developed by Buss and Durkee,<sup>6</sup> designed to measure "a readiness to explode with negative affect at the slightest provocation."

3. Spielberger State Anxiety Scale<sup>7</sup>—a 20-item self-report scale designed to measure state anxiety, that is, "a transitory emotional state characterized by conscious feelings of tension and subjective awareness of heightened autonomic nervous system activity."

*Global rating scales*

4. Moos Menstrual Distress Questionnaire Form T<sup>8</sup>—a 47-item self-report questionnaire of menstrual and premenstrual symptoms.

5. Premenstrual Tension Syndrome Self-Rating Scale<sup>9</sup>—a 36-item self-report questionnaire comprising core symptoms of premenstrual syndrome derived through item analysis of the Moos Menstrual Distress Questionnaire.<sup>8</sup>

As might be expected, the attrition rate of such a demanding protocol was high. Two women declined further participation during the initial control no-treatment month, five others dropped out during placebo treatment months, and a further 11 stopped while receiving progesterone therapy. The reasons for dropping out were varied and included inadvertent pregnancy (1), development of abnormal bleeding (1), deep venous thrombosis while receiving placebo (1), moving out of town (2), need for other medications (3), and dissatisfaction with clinical protocol or response to treatment (8). An additional 10 women were excluded from statistical analysis for various reasons including inability of concurrent records from month 1 to establish a clear premenstrual worsening of symptoms (6), initiation of other psychotropic medications (2), and incomplete records (2). Data from a total of 20 women with concurrently documented severe premenstrual syndrome who completed the full 8-month trial (exceptions for incomplete data: one subject in placebo month 1 and 3 subjects in final control cycle) are analyzed below. Demographic data for this sample are presented in Table I.

**Medication.** Progesterone (200 mg) in a polyethylene glycol base (supplied by Canada Apothecary Limited,

London, Ontario) or an unmedicated polyethylene glycol-based suppository, was self administered twice daily from day 16 (of a 28-day cycle) until onset of menstruation. In women with shorter cycles, treatment was started earlier to coincide with approximately 48 hours after ovulation.

It was impossible to produce placebos identical in color and consistency to the progesterone vaginal suppositories because of the large amount of progesterone incorporated into each suppository. Several types of inert coloring agents were tested before the trial, but the accumulation of these compounds within the vagina precluded their clinical application. To avoid the possibility that some women might differentiate true from placebo suppositories, only women who had never used suppositories previously were included and the consent form was carefully worded to read "I realize I will receive placebo for one or more months during this six month trial" to avoid revealing the precise study design. At the time that crossover of treatments occurred, each subject was advised that there had merely been a slight alteration in the suppository base. No subject, when questioned at the end of the study, correctly identified the time of treatment crossover and most believed that placebo had been administered during only one of the 6 months.

With use of this protocol, medication was administered for a minimum of 12 days in every treatment cycle. The progesterone content and absorption from these polyethylene glycol-based suppositories was examined and compared to other commercially available progesterone suppositories formulated in different free fatty acid bases (K base suppositories, lot No. 9394, or F base suppositories, lot No. 6190, courtesy of Gates Apothecary, Lynnfield, Massachusetts). Progesterone absorption characteristics for each type of suppository were determined by collecting multiple blood samples in the 24 hours following administration of a single suppository to groups of women in the follicular phase of the menstrual cycle. Serum progesterone concentrations were also compared in four women receiving polyethylene glycol-based suppositories 48 hours before menstruation in both progesterone and placebo treatment cycles to determine whether there was a cumulative effect of the suppositories on serum progesterone levels.

In 17 women, fasting plasma samples were obtained in control, placebo, and progesterone treatment cycles immediately before menstruation to determine whether progesterone at these dosages altered plasma lipoprotein patterns.

**Assay methods.** Spectrophotometry was used to examine the mean ( $\pm$ SE) progesterone content of five progesterone suppositories of each type. Serum progesterone concentrations were determined by means of

radioimmunoassay with an antibody to progesterone conjugated in the 11 position (supplied by Dr. S. S. C. Yen, University of California, San Diego) and charcoal separation of bound and free phases. Interassay and intra-assay coefficients of variation were 9.6% and 6.6%, respectively, with recoveries averaging 60%.

The plasma lipoproteins were separated on a discontinuous salt gradient by ultracentrifugation for 24 hours. Aliquots of each of the three major lipoprotein fractions were saponified (19) and the cholesterol content determined by the phthalaldehyde method essentially as described by Zlatkis and Zak<sup>10</sup> with correction for a decrease of absorbance with time on the spectrophotometric readings.

**Data analysis.** Since some subjects had longer cycles than others and consequently some subjects produced more questionnaire responses, a procedure to obtain a uniform number of observations for each subject was implemented. Data from day 3 and day 6 of each cycle were combined to represent early follicular phase (low estrogen and low progesterone, designated menstrual, or M). Day 9 was chosen to represent the late follicular phase (high estrogen and low progesterone, designated intermenstrual, or I). The choice of a single day for the intermenstrual phase was due to an inevitable variability in menstrual cycle length that occurred during the 8-month trial (observations ranged from 21 to 39 days,  $M = 28.8 \pm 3.8$ ). Day 9 was the single most consistent late follicular phase recording for the sample. The late luteal phase (high estrogen and high progesterone, designated premenstrual, or P) was represented by data from the two final sets of questionnaires obtained 3 days apart and thus spanning the 4 to 6 days before the onset of menstruation.

The data analysis was carried out in two stages: First, a preliminary analysis was carried out on the initial no-treatment control cycle to confirm that the subjects selected did suffer from premenstrual syndrome, with use of the "on-off" definition,<sup>4,9</sup> that is, all subjects selected because of high luteal phase premenstrual tension syndrome scores must have also had an asymptomatic period score of  $<4$  during the follicular phase of the cycle. Also used as criteria to define this periodic cessation of symptoms were an intermenstrual depression score of  $<9$  (that is, not depressed according to norms for the Beck Depression Inventory<sup>9</sup>) and a Moos Menstrual Distress Questionnaire score of  $<70$  (as previously described<sup>4</sup>).

Scores on each of the five rating scales were then characterized for this sample by subjecting each of the five dependent variables, that is, the Beck Depression Inventory, the Moos Menstrual Distress Questionnaire, the Premenstrual Tension Self-Rating Scale, the Spielberger State Anxiety Scale, and the Irritability Scale of the Buss Durkee Hostility-Guilt Inventory to analysis



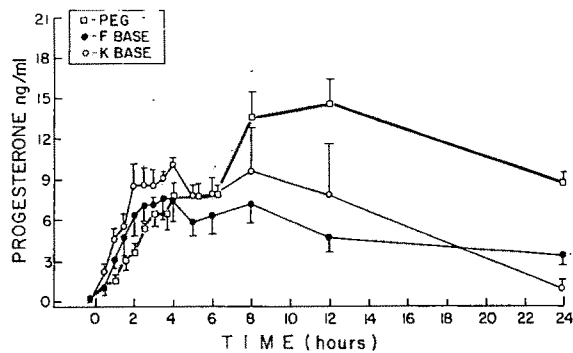


Fig. 1. Mean ( $\pm$ SE) serum progesterone concentrations in female subjects following insertion during the follicular phase of a single 200 mg progesterone vaginal suppository with polyethylene glycol base (PEG,  $n = 7$ ) or free fatty acid bases (F base,  $n = 5$ , and K base,  $n = 5$ ).

of variance for repeated measures with use of the phase of the menstrual cycle as the independent variable.

Subsequent analyses were conducted to examine the efficacy of progesterone therapy. These treatment effects were examined with use of only the late luteal (premenstrual) recordings. Subjects were divided into two groups according to the randomization schedule. Group 1 ( $n = 11$ ) received progesterone in cycles 2, 3, and 4 and placebo in cycles 5, 6, and 7. Group 2 ( $n = 9$ ) received placebo suppositories in cycles 2, 3, and 4 and progesterone in cycles 5, 6, and 7. Cycles 1 and 8 consisted of the initial and final no-treatment control recordings. Analysis of variance for repeated measures was conducted for each group with use of cycle number as the independent variable. Significant main effects were then examined with use of Bonferroni  $t$  tests to determine the treatment conditions responsible for the observed differences.

To evaluate the possibility that the degree of symptom responding was influenced by the order of treatment (that is, group assignment), a further analysis of variance was performed incorporating group designation as a factor.

Finally, data from both groups were pooled according to treatment categories and the mean scores for the three treatment months were compared to those of the three placebo months by means of analysis of variance for repeated measures.

To investigate the possibility that an initial positive response to placebo suppositories might abate with time (thereby revealing a lasting beneficial effect of progesterone) or that a beneficial response to progesterone might only emerge after several treatment cycles, symptom scores were compared during the third treatment cycles for progesterone and placebo, respectively, by means of analysis of variance for repeated measures.

**Table II.** Plasma lipoprotein levels (mean  $\pm$  SE) immediately before menstruation expressed in mg/100 ml of cholesterol in 17 women receiving no treatment (control), 200 mg of progesterone twice daily, or placebo twice daily in the preceding 12 days

Cholesterol fraction	Control	Progesterone	Placebo
Total	208.9 $\pm$ 9.6	209.4 $\pm$ 9.4	218.7 $\pm$ 12.1
Very low-density lipoprotein	27.6 $\pm$ 4.2	27.3 $\pm$ 2.8	28.7 $\pm$ 4.5
Low density lipoprotein	104.9 $\pm$ 8.9	107.0 $\pm$ 8.1	112.6 $\pm$ 0.6
High-density lipoprotein	57.4 $\pm$ 5.3	51.7 $\pm$ 4.1	52.1 $\pm$ 3.8

## Results

**Progesterone content and absorption.** Spectrophotometric analysis of the 200 mg of progesterone in polyethylene glycol-based suppositories used in this trial revealed a progesterone content of  $190.1 \pm 9.1$  mg (mean  $\pm$  SE). This compared favorably with the actual progesterone content of other commercially available 200 mg progesterone suppository preparations from the United States using an F base ( $157.5 \pm 1.8$  mg) and a K base ( $186.9 \pm 11.0$  mg).

Comparison of progesterone absorption with use of 200 mg of progesterone in three different suppository bases revealed that serum levels achieved with polyethylene glycol-based suppositories ( $7.6 \pm 0.9$  ng/ml) were comparable to those attained with F- and K-based suppositories at 6 hours (Fig. 1). Serum progesterone concentrations resulting from polyethylene glycol-based suppositories continued to rise, peaking at  $14.6 \pm 1.9$  ng/ml by 12 hours with a significant residual of  $8.8 \pm 0.9$  ng/ml by 24 hours. In contrast, both F-based and K-based suppositories were associated with lower serum progesterone levels at 12 hours ( $4.7 \pm 1.1$  and  $7.9 \pm 3.9$  respectively) and lesser residual at 24 hours (Fig. 1).

Serum progesterone concentrations, measured in four women 48 hours before menstruation during progesterone and placebo treatment months, were  $19.1 \pm 2.0$  ng/ml and  $4.6 \pm 0.7$  ng/ml, respectively ( $p < 0.0001$ ).

**Plasma lipoprotein analysis.** Plasma lipoprotein patterns showed no significant differences between control (no treatment), progesterone, and placebo cycles (Table II). The values reported here are in keeping with values previously reported for normal women at random times in the menstrual cycle.

**Preliminary analysis.** As expected, based on subject selection criteria, the preliminary analysis confirmed that the sample of subjects were women with primary

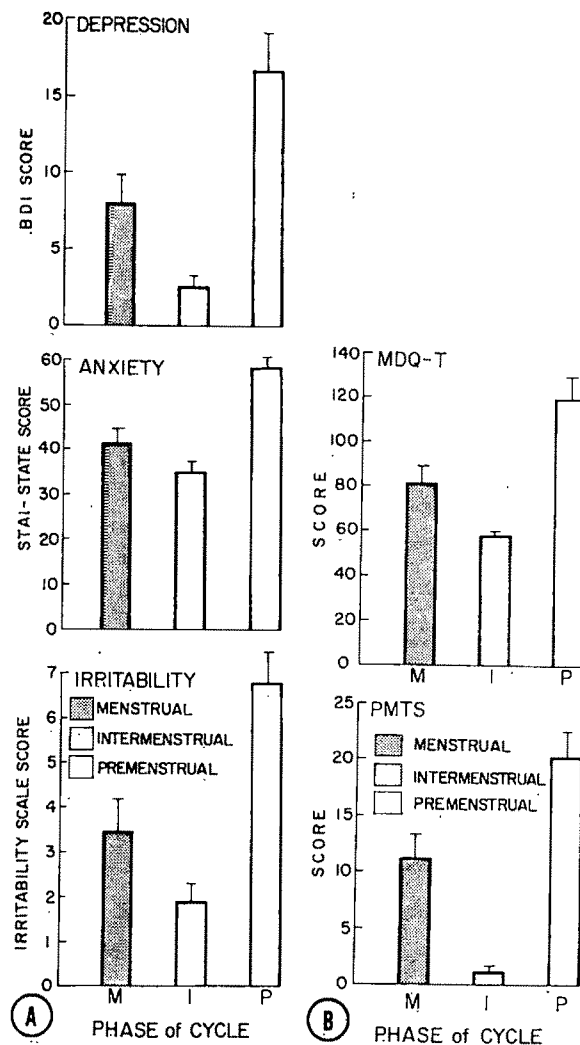
premenstrual syndrome. When data were subjected to analysis of variance for repeated measures, significant main effects for cycle phase ( $p < 0.0001$ ) were observed for each of the five rating scales. Bonferroni  $t$  tests revealed that the pattern of responding was the same for each measure. All responses were most elevated premenstrually and were significantly decreased in the intermenstrual phase. Scores for the menstrual phase were between the scores for the other two phases (Fig. 2).

The severely raised premenstrual symptoms fell dramatically during the intermenstrual phase, demonstrating the crucial "on-off" nature of the disorder. For example, the women in this study were found to be moderately depressed premenstrually (Beck Depression Index:  $16.8 \pm 0.7$ )<sup>5</sup> with premenstrual state anxiety ( $58.6 \pm 2.5$ ) and Moos Menstrual Distress Questionnaire scores ( $120.0 \pm 9.0$ ) equivalent to those previously documented in women with severe premenstrual syndrome.<sup>1</sup> In contrast, the intermenstrual recordings revealed the subjects to be unstressed ( $35.0 \pm 2.1$ ) and to have Moos Menstrual Distress Questionnaire scores ( $58.2 \pm 1.7$ ) comparable to those observed in samples of normal women.<sup>7,8,11</sup> A similar decline in ratings for the Premenstrual Tension Syndrome Scale ( $20.3 \pm 2.0$ ) and the Irritability Scales ( $6.8 \pm 0.6$ ) between the premenstrual and intermenstrual phases again confirmed the presence of a time-limited premenstrual disturbance.

**Treatment effects.** The response to progesterone therapy was initially evaluated by creation of a table on which the mean scores for each rating scale over the 8 months were ranked and compared with the known order of treatment for each group (Table III). If progesterone had been an effective treatment for premenstrual syndrome, then the expected results would have been as follows: for group 1 the recordings obtained for the treatment cycles 2, 3, and 4 would have been significantly lower than those obtained for the placebo cycles 5, 6, and 7 and results for group 2 would have been the reverse. From Table III it can be seen that the expected results were not borne out.

When subjected to analysis of variance, significant main effects for cycle number were observed for certain dependent variables for each group. Post hoc Bonferroni  $t$  test comparisons revealed that the treatment and placebo responses did not differ from each other but that the significant main effects for cycle were the result of consistently higher response scores in the first no-treatment control month (asterisks in Table III).

The possibility that order of treatment (that is, group assignment) influenced symptom reporting was excluded by a subsequent analysis of variance with groups as an independent variable and therefore



**Fig. 2.** Mean ( $\pm$  SE) scores from concurrent records for control month one indicating levels of (A) depression (Beck Depression Inventory, *BDI*), anxiety (Spielberger *STAI-state*), and irritability (Buss Durkee Irritability Scale) and (B) overall symptoms (Moos Menstrual Distress Questionnaire—Form T, *MDQ-T*, and Premenstrual Tension Syndrome Self Rating Scale, *PMTS*) at different phases of the menstrual cycles for the sample of 20 women used to analyze treatment effects.

data were pooled according to treatment for further analysis.

Analysis of variance of the pooled data revealed no significant differences between the responses to progesterone and placebo in any treatment cycle (Fig. 3). In particular, it should be noted that no significant differences were observed in the responses on each of the five rating scales for the third treatment cycles with progesterone and placebo (Fig. 3, *hatched bars*).

Although inspection of the symptom ratings displayed in Fig. 3 gives the appearance of a beneficial effect for both treatments, the clinical relevance of this is doubtful. Evaluation of the transverse mean

**Table III.** Ranked mean scores for each questionnaire and corresponding cycle number

<i>Beck Depression Inventory</i>		<i>Moos Menstrual Distress Questionnaire</i>		<i>Premenstrual Tension Syndrome</i>		<i>Anxiety Scale</i>		<i>Irritability Scale</i>	
<i>Score</i>	<i>Cycle No.</i>	<i>Score</i>	<i>Cycle No.</i>	<i>Score</i>	<i>Cycle No.</i>	<i>Score</i>	<i>Cycle No.</i>	<i>Score</i>	<i>Cycle No.</i>
Group 1 (n = 11)									
17.1	1	115.0	1	19.6	1	59.2	1	6.5	1
14.2	8	110.4	9	17.3	(4)	52.9	8	6.1	8
12.5	(4)	101.8	(4)	16.8	8	50.7	(4)	6.0	(4)
11.2	(3)	100.3	(3)	15.4	(3)	48.1	(3)	4.9	5
11.2	5	98.4	7	14.4	7	48.1	7	4.8	(3)
11.0	7	94.0	5	13.5	5	47.7	5	4.7	(2)
9.6	6	88.1	6*	12.0	6*	46.1	(2)*	4.5	7
9.4	(2)	84.9	(2)*	11.2	(2)*	46.0	6*	3.7	6
Group 2 (n = 9)									
16.4	1	125.1	1	21.2	1	57.8	1	7.5	1
13.1	4	112.6	8	16.7	4	50.7	(6)	6.1	4
12.5	2	101.8	4	15.1	2	48.9	4	5.0	8
11.7	8	98.4	2	14.6	8	48.6	8	5.0	(6)
10.8	(6)	96.1	(5)	14.0	(6)	48.2	(5)	4.9	(5)
10.8	(5)	93.8	(6)	13.0	(5)	45.7	2*	4.0	2*
8.3	(7)	88.3	(7)*	11.7	3	44.6	(7)*	3.6	3*
8.2	3	86.3	3*	9.7	(7)*	43.1	3*	3.4	(7)*

Progesterone months are in parentheses. Treatment schedule: group 1, progesterone months 2, 3, 4 and placebo months 5, 6, 7; group 2, placebo months 2, 3, 4 and progesterone months 5, 6, 7.

\*Significantly different from cycle 1 ( $p = 0.05$ ).

scores across 3 months of each treatment reveals that subjects were still mildly depressed during the treatment and placebo cycles (Beck Depression Inventory treatment,  $10.6 \pm 1.2$ ; placebo,  $10.9 \pm 1.1$ ). Similarly, transverse means of premenstrual scores on the Moos Menstrual Distress Questionnaire (treatment,  $94.3 \pm 4.5$ ; placebo,  $94.4 \pm 4.4$ )<sup>8, 11</sup> and premenstrual State Anxiety ratings (treatment,  $48.1 \pm 1.8$ ; placebo,  $46.6 \pm 1.5$ ) were higher than those obtained in normal samples.<sup>7, 8, 11</sup> As yet, there are no normative data for the full 36-item Premenstrual Tension Syndrome Self-Rating Scale or the Irritability Scale, but recordings for both treatment and placebo cycles in these measures suggested more than minimal discomfort. In effect, the administration of progesterone or placebo did little to alleviate the depression, anxiety, irritability, or physical manifestations of premenstrual syndrome for this sample of subjects.

### Comment

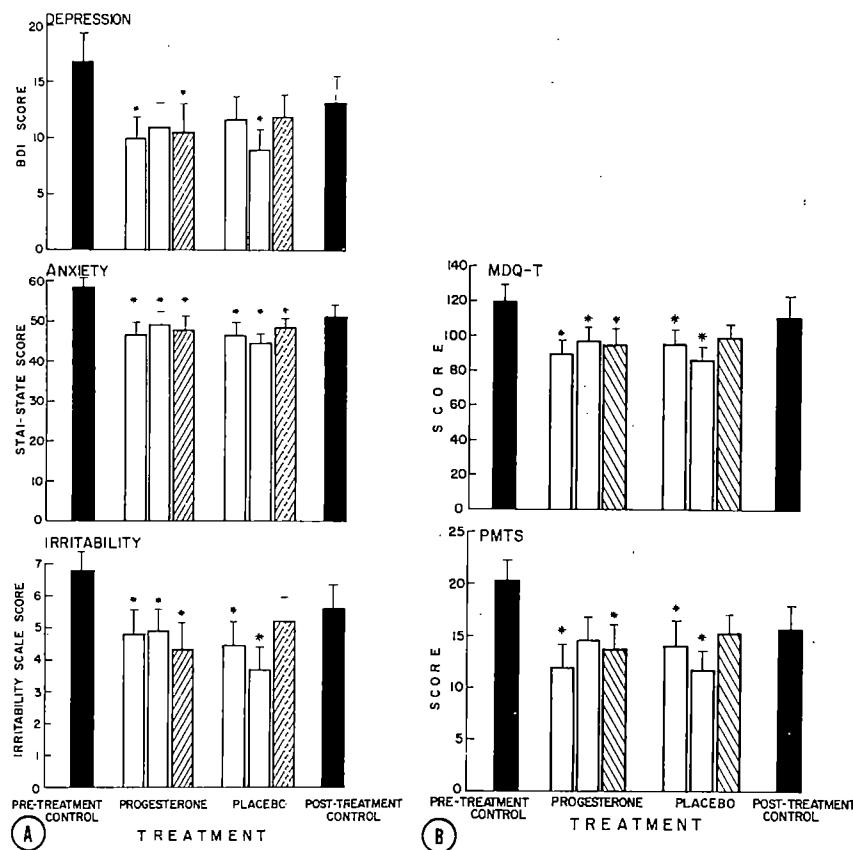
Progesterone therapy for premenstrual syndrome remains controversial. Testimonials to its effectiveness abound, and the lay press in many instances had led women with premenstrual syndrome to believe that progesterone therapy is their right. Attitudes of physicians involved in the treatment of women afflicted by premenstrual syndrome toward progesterone therapy vary from blind acceptance to total condemnation. Many premenstrual syndrome clinics have continued to offer progesterone therapy while awaiting the results

of definitive clinical trials with the view that it may be helpful and is unlikely to be harmful.<sup>5</sup>

Patients are often advised to adjust their own dosage of progesterone to achieve relief of symptoms. As a result, patients may use 2400 mg/day<sup>12, 13</sup> or more, without adequate consideration of the actual plasma concentration achieved or the potential adverse effects of this medication on such factors as serum lipids. Some patients exhibit apparent withdrawal symptoms when attempting to discontinue long-term use of progesterone at these dosages.

Uncontrolled clinical trials that report relief from premenstrual syndrome in 80% of women receiving progesterone therapy<sup>12, 15</sup> have been criticized for failing to take into account the high placebo response rate in this condition.

There have been two previous attempts to evaluate progesterone therapy for premenstrual syndrome with a double-blind placebo-controlled study design.<sup>14, 15</sup> In neither study were determinations made to assess the serum levels of progesterone achieved with the chosen dosage form. Smith et al.<sup>14</sup> evaluated the effectiveness of intramuscular progesterone, 50 mg on alternate days from day 19 until menstruation, in a placebo-controlled trial designed to compare the effectiveness of progesterone and spironolactone alone and in combination for the treatment of 14 women with premenstrual depression. Depression ratings revealed no consistent improvement attributable to progesterone therapy. Regrettably, important demographic information, subject



**Fig. 3.** Mean ( $\pm$ SE) scores from concurrent monthly records of 20 women with premenstrual syndrome, indicating levels of (A) depression, anxiety, and irritability as well as (B) overall symptoms (MDQ-T and PMTS) for control, progesterone, and placebo treatment months. Although scores for progesterone and placebo treatment months were significantly lower than scores for the first no-treatment control cycle (asterisk), there were no significant differences between scores of any progesterone or placebo treatment cycles (Bonferroni  $t$  tests,  $p = 0.05$ ). In particular, by the third treatment month there was neither abatement of the response to placebo nor enhancement of the response to progesterone (hatched bars).

selection criteria, associated symptoms, and methods of assessment are lacking in that report, so critical analysis of methods and results is impossible.

Sampson<sup>15</sup> examined the response to progesterone, 200 mg vaginal suppositories twice daily, in 35 women with premenstrual syndrome in a placebo-controlled double-blind crossover design lasting for 2 months. Twenty-four of these women completed a further month of progesterone therapy of 400 mg twice daily. Her results suggested that marginal improvement in symptoms occurred with both progesterone and placebo treatments, but that there was no significant difference between these two treatments. The only criteria used for subject selection and for monitoring treatment effects in Sampson's study was the Moos Menstrual Distress Questionnaire. The use of this instrument as the sole method for monitoring symptoms in a trial to assess treatment of premenstrual syndrome has theoretical concerns because the Moos Menstrual Distress Ques-

tionnaire includes questions about a variety of symptoms that are not those of premenstrual syndrome (such as dysmenorrhea).<sup>1</sup> Protagonists of progesterone therapy have criticized the validity of her patient inclusion criteria and raised the concern that failure to select a pure population of premenstrual syndrome patients might have accounted for the failure of progesterone to outperform placebo. Certainly the brief (1-month) treatment course for progesterone and placebo in this study failed to take into account the known variability in premenstrual syndrome severity from month to month or the possibility that gradual abatement of an early response to placebo in a longer trial might have unmasked a true lasting beneficial effect of progesterone. Sampson<sup>15</sup> concedes that a longer 6-month trial would have been preferable to the short 1-month treatment schedule that she employed, stating, however, that she discarded this study design in the belief that the maintenance of detailed prospective rec-



ords for this duration would have been "a formidable task for anyone." Finally, the degree of psychological support given to subjects in Sampson's study is unclear so that factors other than medication, such as the validating effect of an initial supportive interview or the insight gained through the process of detailed self-monitoring, may have accounted for the marginal improvement in symptoms seen with both progesterone and placebo.

In view of the many anecdotal reports of beneficial effects of progesterone in premenstrual syndrome and the fact that both progesterone and placebo improved symptoms during Sampson's short-term double-blind trial, we sought to determine whether a longer treatment period might unmask a true beneficial response to this medication. Exacting selection criteria, detailed monitoring of treatment effects with well-established psychometric tools, and a minimum of physician-patient interaction provided the basis for the present investigation. As Sampson<sup>15</sup> predicted, attrition rate was high. This may in part be further evidence for the lack of benefit from progesterone therapy at these doses, since many of the women excluded from final analysis either declined further participation because of a perceived lack of benefit or were ultimately excluded because they had sought and received antidepressants from other physicians. Nevertheless, 20 women with clearly documented severe premenstrual syndrome completed the 8-month trial.

The preliminary analysis of data obtained from the first no-treatment control months not only confirmed that the sample of 20 women subjected to data analysis could be considered a "pure" sample of women with premenstrual syndrome but also demonstrated that concurrent records could identify other subjects who, although meeting the initial diagnostic criteria, failed to demonstrate a pattern of symptoms consistent with premenstrual syndrome on longitudinal evaluation.

To evaluate treatment effects, two types of self-report instruments were chosen to elicit detailed concurrent information about symptoms. Two independent and well-validated rating scales—the Beck Depression Inventory and the Spielberger Anxiety Scale—were used to evaluate two major areas of psychological disturbance in women with premenstrual syndrome: depression and anxiety. Despite the lack of extensive normative data, the irritability subscale of the Buss Durkee Guilt Hostility Inventory was a useful measure of the important symptom of premenstrual irritability. The other two scales, Moos Menstrual Distress Questionnaire and Premenstrual Tension Syndrome Scale, were used to provide a more global assessment of overall suffering from physical as well as psychological manifestation of premenstrual syndrome.

Detailed analysis of data derived from these records

revealed that the use of progesterone as a 200 mg polyethylene glycol-based suppository twice daily from shortly after ovulation until menstruation was associated with a marginal improvement in clinical severity of premenstrual syndrome. The fact that symptoms improved equally during placebo treatment—an effect that failed to abate during three successive cycles—suggests that factors other than progesterone were responsible for the observed response. It seems most likely that the mere expectation that vaginal suppositories would be beneficial led to the slight improvement in symptoms. That this was a true placebo effect and not the result of a better understanding of their condition is suggested by the finding that symptoms returned toward pretreatment levels of severity in the final no-treatment control month (Fig. 3).

The dosage form of progesterone employed in this trial was sufficient to achieve sustained luteal phase progesterone levels in the absence of endogenous progesterone production during testing in the follicular phase. Actual concentrations of progesterone achieved during treatment were obviously much higher because of the additive effects of endogenous production and exogenous administration. Serum progesterone levels 12 hours after administration of the polyethylene glycol-based suppository used in this trial were twice as high as those achieved with other commercial preparations (F- and K-based suppositories) and were certainly high enough when administered twice daily during the luteal phase to correct any hypothetical discrepancy in the estrogen/progesterone ratio in women with premenstrual syndrome.<sup>1</sup>

Concern has been raised that the lowered concentrations of high-density lipoprotein cholesterol observed in users of certain oral contraceptives containing high-potency synthetic progestins may contribute to an increased risk of stroke and myocardial infarction. Progesterone is clearly different in many respects from the synthetic progestins and short-term changes in lipoprotein patterns resulting from luteal phase production of this hormone during the normal menstrual cycle are minimal. Limited data, however, are available to support the safety of pharmacologic dose of progesterone in terms of its effects on serum lipoprotein production,<sup>16</sup> and for this reason we compared serum lipoprotein levels during different treatment phases of this trial. The absence of significant changes in serum lipoproteins at these dosages is reassuring and consistent with previous observations during the menstrual cycle; however, both the safety and efficacy of progesterone administered in higher dosages remains to be established.

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## Eclampsia

### V. The incidence of nonpreventable eclampsia

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The development of eclamptic convulsions is an obstetric complication that is generally considered to be avoidable. The purpose of this study was to investigate the perinatal events associated with 179 consecutive cases of eclampsia, in order to identify specific preventable factors. Based on a careful analysis of medical care received before the onset of eclampsia, the following factors were judged to be at least partially responsible for the failure to prevent eclamptic convulsions: physician error ( $n = 65$ ), magnesium sulfate failure ( $n = 23$ ), late-onset eclampsia ( $n = 22$ ), early onset ( $<21$  weeks) preeclampsia/eclampsia ( $n = 5$ ), abrupt onset eclampsia ( $n = 32$ ), and failure of patient to start prenatal care before the onset of eclampsia ( $n = 34$ ). Fifty-six (31.3%) of the patients received obstetric care that met or exceeded all current standards for delivery of obstetric services. These cases were classified as "unavoidable." (*Am J Obstet Gynecol* 1986;154:581-6.)

**Key words:** Eclampsia, prevention of eclampsia, antecedent factors

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The development of eclamptic convulsions is an obstetric complication that is generally considered to be avoidable.<sup>1</sup> In fact, most authorities believe that improvements in obstetric care could prevent virtually all cases of eclampsia.<sup>2</sup> With this belief in mind, we instituted an intensive effort to improve the quality of obstetric care for preeclamptic patients within our region.

However, although our efforts resulted in improved medical care, this was not reflected in any significant decrease in the incidence of eclamptic convulsions among our patients.

To investigate this problem, we conducted a detailed analysis of prospectively collected data on 179 consecutive cases of eclampsia managed at E. H. Crump Women's Hospital and Perinatal Center over the past 7½ years. The analysis focused on those factors that possibly contributed to the failure to prevent eclamptic convulsions in these women. The investigation included a careful assessment of both the quantity and the quality of medical care that each patient received before the onset of eclamptic convulsions. In addition, we reviewed each chart to determine the degree of patient compliance with medical advice. This paper describes the major findings of our investigation, and discusses specific measures that might be applied clinically to reduce the occurrence of eclampsia in our patient population.

### Material and methods

From August 1, 1977, to March 31, 1985, 179 cases of eclampsia were treated at the E. H. Crump Women's Hospital and Perinatal Center of the City of Memphis Hospitals and the University of Tennessee College of Medicine. The Perinatal Center serves as a tertiary referral center for hospitals, with approximately 30,000 deliveries annually in the Midsouth (Tennessee, Arkansas, Mississippi, Missouri, and Kentucky). A toll-free telephone line is available 24 hours daily for free consultation with an in-house perinatologist and neonatologist.

The chart of each patient was studied to delineate any antecedent prenatal, intrapartum, or postpartum event leading to the development of eclampsia. The information was collected at the time of the diagnosis of eclampsia or when the patient was admitted to the Perinatal Center. Every effort was made to obtain all prenatal, emergency room, and hospital records of all 67 patients referred to the Perinatal Center.

The seventeenth edition of *Williams' Obstetrics* was considered to be the standard in deciding the adequacy of prenatal care and in defining and managing preeclampsia and eclampsia.<sup>3</sup> The assessment of adequacy of care was done retrospectively. For the sake of clarity the 179 cases reported in this study were organized into discrete groups based on the factors judged to be responsible for the failure to prevent eclamptic convulsions. The following terminology was used to define these various factors:

**Patient failures.** Patients who did not seek prenatal care or did not keep their scheduled prenatal care appointments were classified as patient failures.

**Physician error.** The physician error group included

cases in which prenatal care was considered inappropriate and ones in which the physician failed to hospitalize or treat patients with the signs and symptoms of preeclampsia.<sup>4</sup> The quality of prenatal care was considered inappropriate if the patients were given inappropriate return visits by their physician, if maternal blood pressure or urine was not checked at the time of prenatal visit, and if a significant rise in maternal blood pressure was ignored.

**Abrupt-onset eclampsia.** Abrupt-onset eclampsia was the term ascribed to patients who developed convulsions before their next appropriately scheduled prenatal visit or patients who developed convulsions even though they were normotensive following hospitalization for mild preeclampsia remote from term.<sup>5</sup>

**Late-onset eclampsia.** Late-onset eclampsia refers to patients who developed convulsions at or beyond 3 days after delivery.<sup>6</sup>

Mean arterial blood pressure was calculated and recorded for each patient at every prenatal visit, and the average of these recordings between weeks 18 to 26 of gestation was calculated. An arterial pressure level of >85 mm Hg during those weeks was considered abnormal, while a level of <85 mm Hg was considered a reassuring sign that eclampsia would not develop.<sup>7</sup>

All cases of preeclampsia-eclampsia that were managed at the Perinatal Center were treated with intravenous magnesium sulfate (1 part magnesium sulfate to seven parts water, United States Pharmacopeia) as described in a previous report.<sup>4</sup> Some of the cases managed by local physicians were treated with the standard intramuscular regimen recommended by Pritchard.<sup>3</sup>

Gestational age was determined by dates of last menstrual period, early obstetric examinations (uterine pelvic examination, fundal height, date of quickening, and first fetal heart tones identified by fetoscope), and ultrasound examination when available. When this obstetric dating was considered unreliable, the Dubowitz gestational age assessment<sup>8</sup> was used to date the pregnancy.

### Results

During the study period there were 179 patients with eclampsia among 54,300 deliveries (1 per 303 deliveries). Seventy-three of these patients had prenatal care at our clinics. During that time 41,500 patients received prenatal care at the clinics, which means the eclampsia rate among these registered patients was 1 per 568 deliveries. Table I summarizes the clinical characteristics in the 179 patients studied. The mean age ( $\pm$ SD) was  $18.3 \pm 4.6$  years (range, 11 to 36 years), but 158 patients (88%) were younger than 25 years of age.

Fig. 1 outlines the gestational age at time of onset of convulsions. It is interesting to note that eight patients

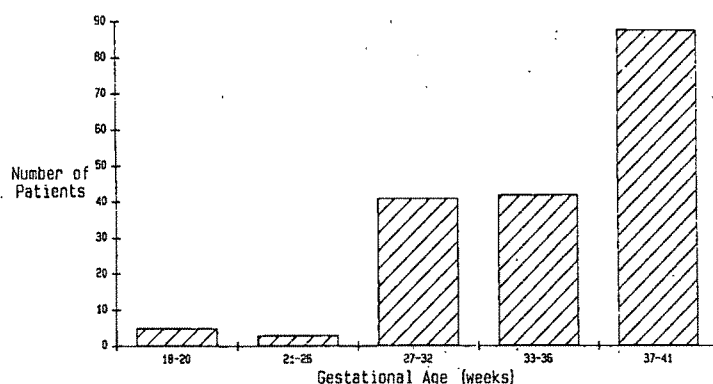


Fig. 1. Gestational age at time of onset of convulsions.

(4.5%) developed eclampsia during the midtrimester, and five of them developed eclampsia before 21 weeks' gestation.

The onset of convulsions occurred before delivery in 130 patients (73%) and after delivery in 49 (27%). Twenty-seven (55%) of the 49 patients with postpartum eclampsia developed convulsions within 48 hours after delivery; the other patients had onset of convulsions 3 or more days after delivery. Eighty-five patients (47%) developed eclamptic convulsions after admission to a hospital and while under medical supervision.

The average mean arterial pressure during the second trimester was calculated for the 118 patients for whom this information was available. Only 26 of these 118 patients (22%) had an average mean arterial pressure >85 mm Hg.

Fig. 2 summarizes the factors antecedent to the development of eclampsia in these 179 patients. To assess the relative contribution of various factors to the development of eclamptic convulsions, the cases were categorized into three discrete groups based on the quality of prenatal care. Then the cases within each group were subdivided based on the specific factors most likely responsible for the failure to prevent the onset of eclamptic convulsions. The definitions for each of these specific factors are detailed in the following paragraphs.

**Patient failure.** Thirty-nine patients did not seek any prenatal care, and an additional 15 patients failed to keep their last scheduled prenatal appointment. In all, patient failure might have been a factor in the development of preeclampsia-eclampsia in 54 patients (30% of the total). Thirty-four of these 54 patients presented with eclampsia, while the remaining 20 patients presented for medical care with the diagnosis of preeclampsia.

**Physician error.** The quality of prenatal care was considered inappropriate in 25 (20%) of the patients who had prenatal care. Twenty-two of these 25 patients were under the care of local physicians, while the other three were patients at our clinics. The quality of pre-

Table I. Clinical characteristics

	n	%
Gravidity		
Primigravid	152	85
Gravidity > 1	27	15
Race		
Black	142	80
White	36	20
Other	1	0
Place of prenatal care		
None	39	23
Our clinics	73	40
Local physicians	67	37

natal care was considered appropriate in the remaining 100 patients. Physician errors were responsible for the development of eclampsia in 35 (35%) of these patients. These errors included either misdiagnosis of preeclampsia ( $n = 22$ ) or failure to institute appropriate therapy once the diagnosis of preeclampsia was established ( $n = 13$ ).

**Failure of magnesium sulfate therapy.** Twenty-three women developed eclamptic convulsions while receiving standard magnesium sulfate therapy. Three patients were receiving the standard intramuscular regimen recommended by Pritchard. The other 20 were receiving intravenous magnesium sulfate; serum magnesium levels were in the "therapeutic range" (4.8 to 8.4 mg/dl)<sup>3</sup> in 11 of these patients and below the therapeutic range in the other nine patients. Some of these patients were receiving the standard intravenous regimen with maintenance doses of 1 to 3 gm/hour. Seven of the above 23 patients had appropriate prenatal care, appropriate management of preeclampsia, and adequate serum magnesium levels at time of convulsions.

**Unavoidable cases of eclampsia.** Table II summarizes the findings in 56 patients (31%) in whom the development of eclampsia was judged unpreventable according to the present standards of prenatal care, definition, diagnosis, and recommended management of preeclampsia. All 56 had appropriate prenatal



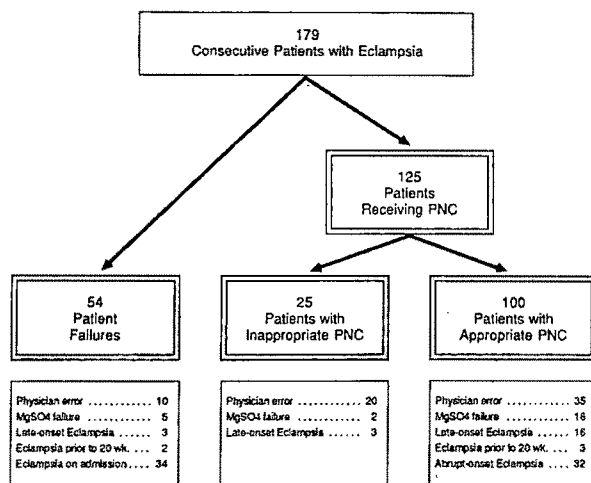


Fig. 2. Summary of factors leading to onset of eclampsia in patients studied. PNC, Prenatal care.

care, were diagnosed and hospitalized appropriately, and were adequately treated with magnesium sulfate. Thirty patients developed abrupt onset of convulsions. Twenty-four of them developed convulsions either before their next prenatal visit ( $n = 22$ ) or in the immediate postpartum period ( $n = 2$ ). The mean interval from last prenatal visit to occurrence of convulsions in the 22 patients with antepartum eclampsia was 8.9 days (range, 3 to 15 days). Ten of the 22 developed convulsions within 1 week after the last visit, and for the others onset was more than 7 days later. All of these patients were given appropriate return visits, and none had any premonitory signs or symptoms of preeclampsia at the time of the last prenatal visit during labor. The other six patients were hospitalized with the diagnosis of mild preeclampsia remoted from term. All patients had normal hematocrit, platelet counts, and liver enzymes initially and on follow-up during hospitalization. They had good response with diuresis and decrease in blood pressure within 48 hours of hospitalization. However, they developed convulsions in the hospital 4 to 10 days following admission.

### Comment

Management schemes designed to prevent eclampsia will succeed or fail, in large part, based on the degree to which they accomplish two tasks: (1) early identification of the patient at risk, and (2) proper preventive therapy once the patient at risk is identified.<sup>2</sup> Three assumptions are made when attempts to prevent eclampsia are undertaken.<sup>2</sup> First, we accept that those factors we list as sensitive and early identifiers of the patients at risk are all inclusive, that is, patients destined to develop eclampsia have prodromal clues that, with a proper index of suspicion, can be identified. Second, it is assumed that appropriate and timely standard pre-

Table II. Reasons for the 56 unav.able cases of eclampsia

Reason	No. of cases
Abrupt onset	24
Onset at 18-20 wk	3
Late postpartum onset	16
Convulsions during magnesium sulfate therapy*	7
Mild pregnancy-induced hypertension with good response†	6

\*Adequate serum magnesium levels.

†Normotensive following hospitalization.

ventive therapy will avoid eclampsia in virtually all patients. Third, the postulate that eclampsia is largely preventable, a priori, is accepted.

While clinging to the belief that eclampsia is largely preventable, we are concerned that current obstetric teaching<sup>3</sup> may be too restrictive and narrow in its definition and description of eclampsia and its prodrome, resulting in an improperly low index of suspicion for the atypical patient. Consequently, proper therapy is often delayed, resulting in increased maternal and perinatal morbidity and mortality.

It is now accepted that pregnancy-induced hypertension has an insidious onset with hypertension appearing relatively late in its course. Changes of clinical significance may occur weeks before clinically detectable hypertension. Some of these changes are subtle and often missed by the nurse or physician during routine prenatal visits.

The current edition of *Williams' Obstetrics*<sup>3</sup> recommends that all pregnant patients be seen every 2 weeks from 28 to 36 weeks' gestation, followed by weekly visits until term. In addition, the definition of preeclampsia requires the presence of hypertension with proteinuria and/or edema after the twentieth week of pregnancy. It is further recommended that magnesium sulfate be used for 24 hours after delivery in the management of preeclamptic patients, since postpartum eclampsia rarely occurs beyond 24 hours after delivery.

Zuspan<sup>2</sup> states that all severe forms of preeclampsia should be preventable by appropriate prenatal care, and once a patient with preeclampsia is hospitalized, convulsions should not occur. In addition, he states that he has "never seen a patient who has been adequately treated with magnesium sulfate have a seizure during the infusion of the intravenous drug." To explain the occurrence of eclampsia, he cites failure of disease recognition in its early phases, failure to increase surveillance of at-risk patients, and inconsistent or inadequate therapy once preeclampsia is identified. In addition, he notes the low incidence of severe preeclampsia/eclampsia in pregnant women who were cared for at military

hospitals (they have frequent prenatal visits and are hospitalized early in the disease). Similar findings were reported by Gilstrap et al.<sup>5</sup> who demonstrated that eclampsia was rarely encountered in patients who received adequate prenatal care and in those who had early and prolonged hospitalization for mild pregnancy-induced hypertension.

In studying the possible factors leading to the development of eclampsia in 66 patients, Campbell and Templeton<sup>9</sup> suggested that in 28 patients (42.4%) eclampsia was not preventable. In addition, they suggested that adequate prenatal care will not prevent most cases of eclampsia, since 71% of cases developed while the patient was under medical and nursing supervision. Inadequate diagnosis and sedation were responsible for development of eclampsia in 26 patients (39.4%). However, they found that 10 patients developed convulsions in spite of adequate sedation.

The results of this investigation and the similar findings of Campbell and Templeton<sup>9</sup> suggest that appropriate prenatal care will not prevent most cases of severe preeclampsia/eclampsia. In fact, 47% of patients in this series developed convulsions following admission to a hospital.

Some authors<sup>7, 10, 11</sup> have suggested the use in the primigravid patient of mean arterial pressure levels during weeks 18 to 26 to identify those patients destined to develop preeclampsia. Page and Christian<sup>10</sup> reported that such levels of >85 mm Hg in a primigravid woman gave her a 1.7 times greater risk of developing preeclampsia than a similar patient whose level was <85 mm Hg. Oney and Kaulhausen<sup>7</sup> calculated the mean arterial pressure level (18 to 25 weeks' gestation) in 200 nulliparous women. The level was  $\geq 90$  mm Hg (positive test result) in 85 women (42%), but only 27 (32%) of these women developed preeclampsia. The remaining 115 patients had a level of <90 mm Hg (negative test result), but 113 of these women (98%) remained normotensive throughout pregnancy. We concluded that this test has a high predictive value when negative. However, in our present series, 78% of the eclamptic patients had mean arterial pressure levels of <85 mm Hg during weeks 18 to 26. Thus the level of the mean arterial blood pressure during the second trimester is a poor predictor for the future development of eclampsia and might give the clinician a false sense of security when it is negative (<85 mm Hg).

Other investigators<sup>11, 12</sup> have suggested screening primigravid patients at 28 to 32 weeks' gestation with the supine pressor test as a means to identify patients at risk for preeclampsia. The accuracy of the supine pressor test has been challenged by several investigators,<sup>13</sup> and even its proponents admit the significant occurrence of false negative and false positive tests. Several

patients in this study developed eclampsia before 28 to 32 weeks' gestation, and hence this test is not helpful in this regard. In addition, the abrupt onset of eclampsia in 24 patients in this study points to the absence of antecedent mild preeclampsia during routine prenatal visits. The opportunity for intervention in such patients is limited. Increasing the frequency of prenatal visits in every primigravid patient at our institution to identify such patients would be impractical from an economic, time, and patient convenience standpoint.

Twenty-three patients developed convulsions while receiving magnesium sulfate. Serum magnesium levels were not determined for three of these patients. In the remaining 20 patients, nine had subtherapeutic and 11 had therapeutic serum magnesium levels. Although it appears appropriate to criticize the management of those patients with subtherapeutic levels, it is important to emphasize that all such patients received standard regimens of magnesium sulfate as recommended for the prevention of eclamptic convulsions. In addition, it is clear that attaining adequate serum magnesium levels is not protection in all such patients. Our experience suggests that about 0.3% of preeclamptic patients will develop convulsions while receiving magnesium sulfate therapy. Similar findings have been reported by other investigators.<sup>14, 15</sup>

Eclamptic seizures first occurred in the postpartum period in 27% of the patients studied. Of these, 22 of 49 patients had onset of convulsions more than 48 hours after delivery. The high incidence of late postpartum eclampsia (12%) in the present series suggests that current obstetric teaching understates this risk and incorrectly lowers the index of suspicion for its occurrence. The reported persistence of abnormalities for 1 to 6 weeks after delivery in preeclamptic and eclamptic patients suggests an increased risk for the development of convulsions throughout this time period.<sup>16</sup>

Sixty-nine percent of the 179 cases of eclampsia reported in this series were judged to be potentially preventable. In each of these cases, poor patient compliance and/or nonstandard patient management may have contributed to the eventual occurrence of eclamptic seizures. Efforts to improve patient access to, enrollment in, and compliance with prenatal care, in concert with more rigid adherence to published therapeutic recommendations, would be expected to lower the risk of eclampsia for such patients. However, modifications of current obstetric teachings, designed to heighten awareness of the risk of both midtrimester and late postpartum eclampsia, could further reduce its occurrence. Short-term postpartum anticonvulsant therapy may also be of benefit in certain cases. However, randomized clinical trials are needed to prove the value of extending the duration of this anticonvulsant therapy. The risk of eclampsia both during conservative

therapy of mild disease and in patients receiving appropriate magnesium sulfate therapy may require reassessment of current obstetric practices.

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## Variable decelerations during nonstress tests are not a sign of fetal compromise

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An examination of 908 fetal heart rate tests of 418 consecutive patients revealed brief variable decelerations in more than 50.7% of the patients. Although an association existed with nuchal cord location found at delivery, no association existed between these variable decelerations and fetal heart rate decelerations during labor, low Apgar scores at birth, or birth weight. We find no evidence to suggest that these brief variable decelerations are a sign of fetal compromise or an indication for obstetric intervention. (*AM J OBSTET GYNECOL* 1986;154:586-90.)

**Key words:** Fetal heart rate, deceleration, nonstress tests

In the management of women with high-risk pregnancies, surveillance of fetal well-being by means of antepartum fetal heart rate tests has attained widespread use. A number of authors<sup>1-6</sup> have found that a

reactive fetal heart rate pattern is predictive of a successful pregnancy outcome, whereas a nonreactive test, especially if accompanied by late decelerations of fetal heart rate, is associated with increased likelihood of fetal distress during labor, neonatal morbidity, and perinatal death. Recently several authors<sup>7-15</sup> have directed attention to the significance of variable decelerations encountered during antepartum fetal heart rate tests and suggested that the occurrence of this pattern is a potentially ominous sign and is associated with abnormal umbilical cord location, oligohydramnios, impaired fetal growth, and increased risk to the fetus. We

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**Table I.** Indications for antepartum fetal heart rate testing

Indication	No. of patients
Preeclampsia	100
Postdates	93
Chronic hypertension	60
Diabetes mellitus	37
Suspected poor fetal growth	25
Decreased fetal movement	21
Poor past-pregnancy history	16
Other	73
Not stated	207

**Table II.** Occurrence of fetal heart rate deceleration in any of last four antepartum fetal heart rate tests before delivery (N = 428)

Deceleration	%
Brief, $\leq 15$ sec (V-shaped)	45.8
Longer, $> 15$ sec (U-shaped)	14.3
Combined (V- + U)	50.7

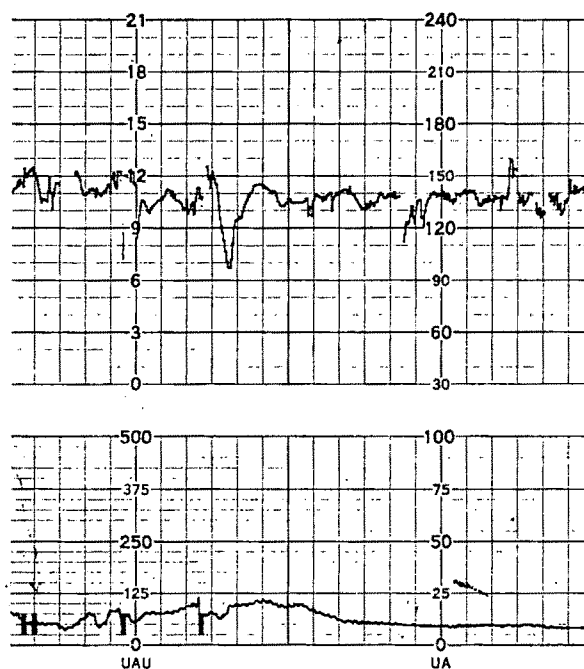
performed the following study in an attempt to evaluate the significance of these variable decelerations.

### Methods

To determine the occurrence rate and evaluate the clinical significance of these decelerations, we analyzed the last four antepartum fetal heart rate tests of 428 consecutive patients seen in our antenatal testing laboratory over an 18-month time span (March 1, 1979, to August 31, 1980). A total of 908 tests were examined, since some patients had less than four tests.

The customary protocol in our testing laboratory is to perform antepartum fetal heart rate tests twice weekly. The primary mode of evaluation is the nonstress test. A test is considered reactive with the presence of two or more accelerations of 15 bpm lasting 15 seconds or more in a 20-minute time span. In general, a nonreactive test is followed by an oxytocin challenge test, and this test is considered negative if no late decelerations are seen with a contraction frequency of at least three in 10 minutes. A test was classified as positive if there were consistent and persistent late decelerations in the absence of hyperstimulation. Tests were performed with the patient in the semirecumbent or lateral recumbent positions.

A variable deceleration occurring during a nonstress test was defined as a rapid drop in heart rate of  $\geq 20$  bpm below the baseline rate. The decelerations were classified by duration ( $> 15$  or  $\leq 15$  seconds), by the maximal fall below baseline, and by the number of decelerations in any one test. The presence of variable decelerations was not considered in the clinical management of the patients. Intrapartum fetal heart rate



**Fig. 1.** Example of a V-shaped fetal heart rate occurring in association with a spontaneous contraction and fetal movement.

records were available for 315 of the 428 subjects and were evaluated with use of the criteria of Hon and Quilligan.<sup>16</sup>

To examine the clinical significance of variable decelerations, the occurrence and frequency of these decelerations in the last nonstress test before delivery were compared with the occurrence and frequency of intrapartum fetal heart rate patterns, with 1- and 5-minute Apgar scores and with mode of delivery by means of  $\chi^2$  analysis.

Associations with birth weight and gestational age at delivery were made with use of Student's *t* test.

### Results

The indications for the antepartum fetal heart rate test are shown in Table I. The most common reasons were acute or chronic hypertension. Of the patients, 51.6% had more than one indication.

The occurrence of variable decelerations in the last four antepartum fetal heart rate tests before delivery is indicated in Table II. These decelerations were classified according to their duration into brief (V-shaped) with a duration of  $\leq 15$  seconds as shown in Fig. 1, and longer (U-shaped) with a duration of  $> 15$  seconds, as shown in Fig. 2. Most of the U-shaped variable decelerations had a duration of 16 to 30 seconds, and variable decelerations of extended duration ( $> 30$  seconds) were rarely seen. In the total group of subjects,  $> 50\%$  of the patients had one or more variable decelerations in one of the last four antepartum fetal heart rate tests before



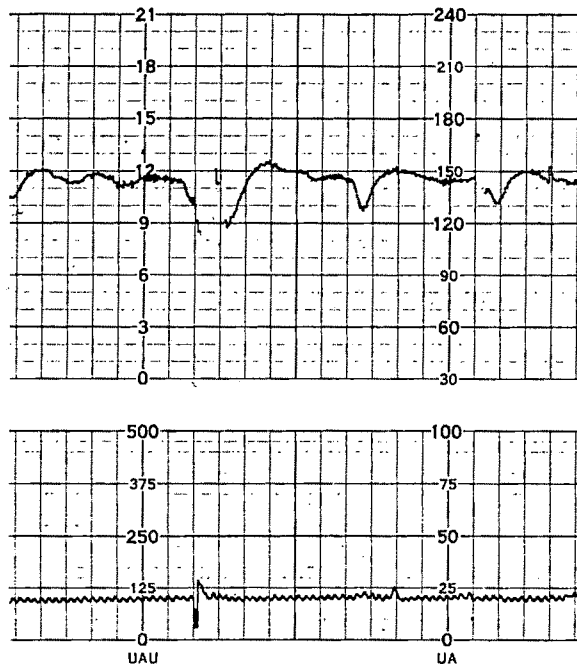


Fig. 2. Example of a U-shaped deceleration associated with fetal movement and a V-shaped deceleration not associated with fetal movement.

delivery. In the last antepartum fetal heart rate test before delivery, as shown in Table III, 31% of the patients had one or more V-shaped decelerations, and 9% had one or more U-shaped decelerations.

In evaluating possible associations of variable decelerations with perinatal events, no difference in significance was found when comparing duration of deceleration (V-shaped versus U-shaped) or in comparing extent of fall in fetal heart rate from baseline. Therefore the presence and number of any variable deceleration in the last antepartum fetal heart rate test before delivery was examined for associations with intrapartum fetal heart rate patterns, Apgar scores, and other perinatal events.

The incidence of periodic fetal heart rate patterns during intrapartum fetal heart rate monitoring is shown in Table IV. No association existed between the occurrence of variable decelerations in antepartum fetal heart rate tests and the presence or number of intrapartum variable, early, or late fetal heart rate decelerations. The data relating to severe variable decelerations and late decelerations are shown in Table V. Likewise, no association was present between the occurrence of these antepartum variable decelerations and the occurrence of low Apgar scores at 1 or 5 minutes after birth, as shown in Table V.

The subset of patients who were  $\geq 42$  weeks in gestational age were examined for the same associations. Again, no association existed between occurrence of variable decelerations and intrapartum fetal heart rate patterns or Apgar scores.

Table III. Incidence of fetal heart rate deceleration in last antepartum fetal heart rate test before delivery (N = 428)

No. of decelerations	Brief (V-shaped) decelerations (%)	Longer (U-shaped) decelerations (%)
0	68.9	90.9
1	13.8	4.4
2	6.6	2.3
3	4.2	1.4
4 or more	6.5	1.0
Total	100	100

Table IV. Incidence of intrapartum fetal heart rate patterns

Pattern	Incidence (%)
Mild variable decelerations	77.7
Severe variable decelerations	5.1
Early decelerations	13.6
Late decelerations	18.6
Accelerations	69.2

The group of patients who exhibited a nonreactive antepartum fetal heart rate test (24% of the group) was considered, and once again, no association existed between variable decelerations and intrapartum fetal heart rate patterns or Apgar scores.

Neither V-shaped nor U-shaped decelerations showed any association with birth weight (as shown in Table VI) or gestational age when patients who exhibited these signs were compared by means of Student's *t* test with patients who did not exhibit them.

An association was found between variable decelerations and umbilical cord abnormalities found at the time of delivery, as shown in Table VII. These abnormalities consisted of 67 patients with a nuchal cord location and one patient with a true knot of the cord. Although a statistically significant association was present, the sensitivity and specificity were only 38% and 70%, respectively.

Two fetal deaths and five neonatal deaths occurred in this series of high-risk patients, for a perinatal mortality of 16.4 per 1000 live births. One fetal death was in a twin gestation before 30 weeks with a positive oxytocin challenge test and brief variable decelerations, in which the other twin showed no sign of fetal compromise and intervention was withheld. The other fetal death occurred in a postdates patient after a nonreactive antepartum fetal heart rate test, which was read as a negative oxytocin challenge test but which in retrospect showed small but clearly present late decelerations. No variable decelerations were present in the fetal heart rate tracing. One neonatal death was that of

**Table V.** Comparison of occurrence and frequency of variable decelerations in last antepartum fetal heart rate test with fetal heart rate patterns in labor and Apgar scores at birth

No. of decelerations	Severe variable decelerations during labor*			Late decelerations during labor†			Apgar score at 1 minute‡			Apgar score at 5 minutes§		
	Absent (n)	Present		Absent (n)	Present		0-6		7-10 (n)	0-6		7-10 (n)
		n	%		n	%	n	%		n	%	
0	186	11	5.6	164	33	16.8	80	29.4	192	20	7.4	252
1	41	2	4.7	36	7	16.3	14	24.1	44	2	3.4	56
2 or more	71	3	4.1	56	18	23.6	29	30.5	66	10	10.5	85
Total	298	16		256	58		123		302	32		393

\* $\chi^2 = 0.28$ ;  $p = \text{NS}$ .

† $\chi^2 = 2.21$ ;  $p = \text{NS}$ .

‡ $\chi^2 = 0.79$ ;  $p = \text{NS}$ .

§ $\chi^2 = 2.21$ ;  $p = \text{NS}$ .

**Table VI.** Comparison of occurrence of variable decelerations in last antepartum fetal heart rate test with birth weight

Decelerations	Birth weight (gm)	
	Mean	SD
Present	2954	890
Absent	2994	766

$t = 0.49$ ;  $p = \text{NS}$ .

an infant with trisomy 13, three were those of infants weight  $\leq 660$  gm, and one involved an 1100 gm infant who suffered perinatal asphyxia and showed severe hyaline membrane disease at postmortem examination. During this same time frame, the perinatal mortality at this hospital for nonreferral patients (residents of Forsyth County) was 17.5 per 1000 live births.

#### Comment

Previously published studies<sup>7-15</sup> have found occurrences of variable decelerations in antepartum fetal heart rate tests varying from 1.6%<sup>11</sup> to 56.8%.<sup>8</sup> An association with abnormal umbilical cord anatomy or location ranges from 45.6% to 92%.<sup>9</sup> This wide variation between studies may be due to differences in the definition of these phenomena, since only three studies<sup>11, 13, 15</sup> offered a precise definition of these decelerations. With use of a definition of variable deceleration as a drop of  $\geq 20$  bpm below baseline rate, we find an occurrence of these patterns in 50.7% of our patients. Of those patients exhibiting these patterns, 24.3% had a nuchal cord location at birth.

The results of this study indicate that these variable decelerations or V- and U-shaped dips in fetal heart rate were frequently encountered in antepartum fetal heart rate tests, with over half of all patients undergoing these tests exhibiting these patterns in one of the last

**Table VII.** Comparison of occurrence and frequency of variable decelerations in last antepartum fetal heart rate test with nuchal cord found at delivery

No. of decelerations	Nuchal cord		%
	Absent	Present	
0	252	42	14.3
1	53	6	13.8
2 or more	54	20	27.0
Total	359	68	

$\chi^2 = 8.86$ ,  $p = 0.012$ , sensitivity = 38.2%, specificity = 70.2%.

four tests before delivery. Although an association existed between these variable decelerations and nuchal cord location at delivery, no association existed between these fetal heart rate patterns and any measurement of intrapartum or neonatal morbidity in the entire group of patients studied, in the group over 42 weeks' gestation, or in the group with a nonreactive antepartum fetal heart rate test. These patterns were not associated with low birth weight infants.

Some authors<sup>7, 11, 14</sup> have suggested that variable decelerations occurring during antepartum fetal heart rate tests are a sign of oligohydramnios and should be considered ominous, since the diminished amniotic fluid volume may represent a compromised fetus or may lead to a cord accident. Although we did not perform ultrasound scans routinely in this series of patients, <10% of these patients showed any clinical sign of oligohydramnios or impaired fetal growth, while >50% of the patients exhibited these fetal heart rate patterns. It seems highly unlikely that oligohydramnios was present in >50% of all patients routinely screened in the Antenatal Testing Laboratory.

Decelerations of the fetal heart rate lasting >60

seconds occurring during antepartum fetal heart rate tests may be of more worrisome significance. Two authors<sup>11, 14</sup> report an association of these prolonged decelerations with fetal distress in labor. In both of these studies the occurrence rate of these decelerations was 1.6% of patients receiving antepartum fetal heart rate tests. These prolonged decelerations were not identified in the antepartum fetal heart rate tests of patients in this study, and variable deceleration of >30 seconds were rarely encountered.

We conclude that if one examines antepartum fetal heart rate tests in a rigorous manner, brief variable deceleration will frequently be seen. These brief variable decelerations should not be confused with the much less common but potentially ominous prolonged variable deceleration (>60 seconds) which may be associated with severe oligohydramnios and fetal compromise. Variable decelerations that are of <30 seconds duration are not a sign of a compromised fetus nor are they an indication for immediate delivery.

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# The effect of antenatal dexamethasone administration on the prevention of respiratory distress syndrome in preterm gestations with premature rupture of membranes

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A prospective blinded randomized study was carried out to determine the effect of antepartum administration of dexamethasone on the incidence of respiratory distress syndrome in 250 patients with gestations between 28 and 33 weeks complicated by premature rupture of membranes. The incidence of respiratory distress syndrome was not lowered by the length of rupture of membranes in the 124 untreated patients. The overall incidence of respiratory distress syndrome was reduced from 51% to 25% by the administration of corticosteroids. Further, the dexamethasone-treated group had a statistical significant reduction in the incidence of intraventricular hemorrhage, total time of hospitalization, and average cost per patient. No statistical difference was encountered in the incidence of maternal or neonatal sepsis. (AM J OBSTET GYNECOL 1986;154:591-5.)

**Key words:** Antenatal dexamethasone, respiratory distress syndrome, premature rupture of membranes

Of all causes of prematurity-related morbidity, respiratory distress syndrome is the most common, with an incidence of about 50% in gestations under 33 weeks and a mortality/case ratio of approximately 15%. In managing expectantly preterm pregnancies with premature rupture of membranes, one is attempting to prolong intrauterine life, which should decrease the incidence of respiratory distress syndrome.<sup>1-3</sup> Unfortunately, about 75% of patients managed expectantly are delivered within 7 days of the onset of rupture of membranes, thus achieving minimal benefit from an expectant management approach.

Although it is likely that in future years respiratory distress syndrome will be treated by artificial surfactant administration to the neonate,<sup>4</sup> the antepartum use of glucocorticosteroids has been proposed to accelerate fetal lung maturation. The benefits of corticosteroids have been studied in several well-designed prospective studies starting with the reports of Liggins and Howie.<sup>5</sup> Although glucocorticosteroid use is a subject of continued controversy, the consensus is that when dealing with preterm gestations with intact membranes, antepartum corticosteroid use does result in a significant reduction in respiratory distress syndrome for gesta-

tions under 34 weeks.<sup>6</sup> The benefit of corticosteroids in preterm gestations complicated by premature rupture of membranes, however, has been studied prospectively in randomized fashion by very few authors and with conflicting results. Garite et al.<sup>7</sup> failed to demonstrate a decrease in the incidence of respiratory distress syndrome by use of corticosteroids in a prospective study involving 160 patients with premature rupture of membranes at between 28 and 34 weeks. On the other hand, Mead and Clapp<sup>8</sup> did report a decrease in the incidence of respiratory distress syndrome by the use of corticosteroids in preterm gestations with premature rupture of membranes, although the 43-patient study was neither randomized nor blinded. The purpose of this study is to establish whether the antenatal administration of corticosteroids results in improved neonatal outcome in gestations with premature rupture of membranes and to determine whether there is increased risk of neonatal and maternal infection.

## Material and methods

Patients admitted to Orlando Regional Medical Center from January 1983 through July 1985 with single uterine gestations between 28 and 33 weeks and premature rupture of membranes were selected for this study. Gestational age was established by menstrual history, initial prenatal examination, and ultrasound report when available. On admission each patient had an ultrasound evaluation to confirm gestational age, placenta location, fetal position, and oligohydramnios. Rupture of membranes was documented by obvious

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**Table I.** Characteristics of both groups of mothers

	No steroid treatment (n = 124)	Steroid treatment (n = 121)	p
Gestational age (wks)	30.07 ± 0.57	30.76 ± 0.42	NS
Maternal age (yr)	22.8 ± 1.1	21.7 ± 0.9	NS
Latency period (days)	3.93 ± 1.26	3.77 ± 1.19	NS
Length of labor (hr)	5.15 ± 1.36	6.17 ± 1.66	NS
No. with tocolysis used (%)	—	44 (36)	
No. of cesarean sections (%)	40 (32)	42 (35)	NS
Noncephalic	18	19	
With fetal distress	13	13	
Repeat	5	5	
Other	4	5	

NS = Not significant.

pooling with sterile speculum and confirmed by ferning and alkaline pH by Nitrazine paper. Aerobic vaginal cultures were obtained on admission. In addition, a rapid screening test (5 to 20 hours) for group B streptococci based on coagglutination methods was used. If group B streptococci were identified, the patient was treated with 1 gm of ampicillin every 6 hours in the absence of evidence of intraamniotic infection. If the patient was allergic to ampicillin, a first-generation cephalosporin was used. The rapid-screening test was repeated in 48 hours to confirm eradication of the carrier state for group B streptococci. A reactive nonstress test was required for gestation of <30 weeks. If non-reactive after 40 minutes, the test was repeated within 24 hours following breakfast, and if the nonreactivity persisted, sonography was performed to verify fetal breathing movements.

Patients in labor at the time of admission, those with foul-smelling fluid, those with discrepancy in gestational age, sonographic evidence of intrauterine growth retardation, or nonreassuring antenatal testing, those with fetal lung maturity confirmed by the presence of phosphatidylglycerol in the amniotic fluid obtained from vaginal pooling, and neonates with congenital anomalies inconsistent with life were excluded from the study.

The eligible patients were then randomized by their medical record number to an untreated group or to a corticosteroid-treated group receiving four doses of 6 mg of dexamethasone intramuscularly 12 hours apart. Both groups of patients were managed expectantly; however, patients in the steroid-treated group of <33 weeks' gestation who were not delivered within 7 days from date of completion of treatment received a second treatment of 24 mg of dexamethasone as previously outlined. Vaginal cultures and a nonstress test were repeated in all patients not delivered within 7 days of admission. The onset of intraamniotic infection, defined by a persistent temperature of 100.4° F in the absence of another source, was monitored by daily

white blood cell count with differential and C-reactive protein titers, oral temperature, and fetal heart rate recorded every 4 hours. Evidence of abdominal tenderness or foul-smelling discharge was sought twice daily. If the clinical diagnosis of chorioamnionitis was made, the patient was started on ampicillin and gentamicin therapy and labor was induced if presentation was cephalic or a cesarean section was performed if presentation noncephalic. The clinical diagnosis of chorioamnionitis was confirmed by histopathologic studies of the placenta.

Patients in the steroid-treated group in labor before the completion of the 48 hours of dexamethasone therapy received intravenous magnesium sulfate for tocolysis in the absence of signs of chorioamnionitis. When magnesium sulfate failed to arrest labor after 1 hour of maximum dosage, 4 gm/hr or a level of 9 mg/dl, ritodrine hydrochloride was added up to a maximum dose of 0.35 mg/min. Tocolysis was discontinued when dexamethasone therapy was completed or when cervical dilatation was assessed to be >4 cm by sterile speculum examination. All infants were monitored continuously through labor. Fetal heart rate patterns were classified as being normal, with persistent variable decelerations, or with persistent late decelerations. Intrapartum fetal distress was diagnosed on the basis of persistent late deceleration or progressively worsening variable deceleration pattern.

Following delivery, the infant was examined by one of three attending neonatologists responsible for assigning Apgar scores and establishing gestational age by Dubowitz scoring. Surface, oropharynx, urine, blood, and cerebrospinal fluid cultures were obtained on admission to the neonatal intensive care unit before starting antibiotic therapy. In addition, a urine Wellcogen latex agglutination test was performed on all neonates for detection of group B streptococci sepsis.<sup>9</sup> The diagnosis of neonatal sepsis was made on the basis of a positive blood or cerebrospinal fluid culture or positive Wellcogen urine screening and/or oropharynx

**Table II.** Characteristics of infants from both groups

	No steroid treatment (n = 124)	Steroid treatment (n = 121)	p
Gestational age (wks)	31.58 ± 0.41	31.18 ± 0.39	NS
Birth weight (gm)	1369 ± 14	1437 ± 15	NS
Apgar score			
1 minute	4.88 ± 1.87	4.98 ± 1.93	NS
5 minutes	7.05 ± 1.65	6.81 ± 2.01	NS
Race			
No. of white (%)	76 (61)	54 (43)	0.05
No. of nonwhite (%)	48 (39)	67 (57)	0.05
Sex			
No. of female (%)	67 (54)	58 (48)	NS
No. of male (%)	57 (46)	63 (52)	NS

NS = Not significant.

**Table III.** Latency period and its effect on respiratory distress syndrome

Latency period (days)	No steroid treatment (n = 124)		Respiratory distress syndrome		Steroid treatment (n = 121)		Respiratory distress syndrome	
	n	%	n	%	n	%	n	%
<1	35	28	19	54	27	22	7	26
1-3	32	26	17	53	41	34	11	27
4-7	27	22	14	52	23	19	5	22
>7	30	24	13	43	30	25	7	23

cultures along with radiographic findings and clinical signs of congenital infection as determined by the attending neonatologist. The diagnosis of respiratory distress syndrome was made by the attending neonatologist on the basis of characteristic radiologic findings along with increasing respiratory requirements. Neither the neonatologist nor the radiologist was aware of the mode of treatment used on the mother. Bedside echoencephalography was performed within 72 hours of birth to detect evidence of intraventricular hemorrhage with use of a portable real-time scanner (ATL MK 300) with a 5 MHz transducer. Hemorrhages were graded according to the system proposed by Papile et al.<sup>10</sup>

Analysis of the data was performed by means of  $\chi^2$  and two-tailed *t* tests. Differences were considered significant at a probability level of 0.05.

## Results

The study consisted of 250 patients with a single fetus between 28 and 33 weeks with premature rupture of membranes; of these, 121 were randomly assigned to the steroid treatment group. There were no cases of intrauterine death. The characteristics of the patients of the two groups, as shown in Table I, demonstrate adequate matching. Similarly, the characteristics of the neonates are shown in Table II.

For the 250 patients the latency periods, defined as the time from rupture of membranes to the onset of

**Table IV.** Effectiveness of tocolysis

Cervical dilatation (cm)	No. of patients	At 24 hours		At 48 hours	
		n	%	n	%
Closed	33	26	79	23	70
1-2	7	5	71	3	43
3-4	4	2	50	—	—
Total	44	33	75	16	59

labor, are summarized in Table III. As shown, expectant management was successful in prolonging intrauterine life >7 days in only 24% of the patients studied. In the remaining patients the effect of rupture of membranes on the incidence of respiratory distress syndrome was studied in the nonsteroid-treated pregnancies. The results summarized in Table III demonstrate that prolonged rupture of membranes alone does not enhance pulmonary maturation in the premature infant.

Tocolysis was required in 44 patients, and the results are summarized in Table IV. As seen, therapy was successful in prolonging pregnancy at least 24 hours in 75% of the cases. Only in three of these patients was ritodrine needed to complement magnesium sulfate therapy. Although there was no increase in incidence in maternal or neonatal infection in the group treated with tocolytic agents, there were, however, two cases of acute pulmonary edema. Both cases occurred after 24 hours on 3 gm/hr of magnesium sulfate alone and re-

**Table V.** Results from antepartum corticosteroid administration

	No steroid treatment (n = 124)		Steroid treatment (n = 121)		p
	n	%	n	%	
Respiratory distress syndrome	63	51	30	25	0.001
Intraventricular hemorrhage					
Total	33	27	13	11	0.01
Grade 3-4	14	11	7	6	NS
Necrotizing enterocolitis	4	3	1	1	NS
Neonatal sepsis	11	9	11	9	NS
Group B streptococci		5		5	
<i>Escherichia coli</i>		2		1	
<i>Hemophilus influenzae</i>		2		2	
Other		2		3	
Neonatal mortality	13	10	7	6	NS
Respiratory distress syndrome		8		3	
Sepsis		3		3	
Intraventricular hemorrhage		2		1	
Chorioamnionitis	18	14	16	13	NS
Neonatal hospitalization (days)	38.07 ± 3.07		22.2 ± 2.8		0.01
Total cost per neonate (\$)	27,600 ± 2080		10,300 ± 850		0.01

**Table VI.** Effect of sex and race on the incidence of respiratory distress syndrome

	No steroid treatment (n = 124)		Steroid treatment (n = 121)		p
	n	%	n	%	
Overall incidence	63	51	30	25	0.001
Gestational age (wk)					
28-30	32/52	61	17/53	32	0.01
31-33	31/72	43	13/68	19	0.01
Sex and race					
Female	27/67	40	11/58	19	0.01
White	16/38	42	7/25	28	NS
Nonwhite	11/29	38	4/33	12	0.05
Male	36/57	63	19/63	30	0.01
White	26/38	68	12/29	41	0.05
Nonwhite	10/19	68	7/34	21	0.05

NS = Not significant.

sponded successfully to use of a positive end-expiratory pressure mask and Lasix therapy. In one of the cases, overhydration was documented before injection of epidural anesthetic. The second patient had a Swan-Ganz catheter inserted, which revealed normal pulmonary artery pressure and cardiac output. No positive fluid balance was recorded in this patient. Thus it is strongly suspected that the cause of this case of pulmonary edema could have been increased permeability of the pulmonary vessels, perhaps an effect of corticosteroids.<sup>11</sup>

The effect of antenatal glucocorticosteroid administration on patients with premature rupture of membranes and single gestations between 28 and 33 weeks is summarized in Table V. The data demonstrate that antepartum dexamethasone administration resulted in

a statistically significant reduction in the incidence of respiratory distress syndrome (51% compared to 25%,  $p < 0.001$ ) and of intraventricular hemorrhage (27% versus 11%,  $p < 0.01$ ) without any difference in the rate of chorioamnionitis and neonatal sepsis. Moreover, the infants from the steroid-treated mothers had substantially less time of hospitalization, 38 versus 22 days, and average cost per patient, \$27,500 versus \$10,300, statistically significant at  $p < 0.01$ . There was no statistically significant difference in the incidence of severe intraventricular hemorrhage, necrotizing enterocolitis, or mortality.

The effect of sex and race on the incidence of respiratory distress syndrome was further studied. As shown in Table VI, although respiratory distress syndrome was found to be less prevalent in the female neonate (40% versus 63%,  $p < 0.05$ ), the use of antenatal corticosteroids resulted in a statistically significant reduction in both the female neonate (19% versus 40%) and the male (30% versus 63%,  $p < 0.01$ ). Similarly, although antenatal dexamethasone administration was particularly effective in protecting nonwhite babies against respiratory distress syndrome (44% versus 16%,  $p < 0.01$ ), its use also resulted in a reduction of respiratory distress syndrome in the white population (55% versus 35%,  $p < 0.05$ ).

### Comment

The merits of an expectant management philosophy in dealing with the preterm gestation with premature rupture of membranes has been well demonstrated by the results of previous studies. Although the risks of infectious morbidity cannot be ignored, for gestations of <33 weeks the incidence of respiratory distress syndrome is at least three times that of neonatal infection.

This study confirms the findings of previous reports that prolonged rupture of membranes does not enhance pulmonic maturation.<sup>12</sup> Further, only 25% of the preterm gestations with premature rupture of membranes have a latency period that exceeds 7 days. Therefore a complementary therapeutic modality should be employed if improved neonatal outcome is to be achieved in these high-risk pregnancies.

This study demonstrates that the antepartum administration of corticosteroids along with tocolysis as needed in pregnancies with premature rupture of membranes with satisfactory reactive nonstress test or biophysical profiles resulted in a significant decrease in the incidence of respiratory distress syndrome without an increased risk of maternal or neonatal infection. Furthermore, the use of corticosteroids also resulted in a substantial reduction of intraventricular hemorrhage, neonatal hospital stay, and cost.

Nevertheless, it is imperative that an expectant management approach continues to be employed. Despite the benefits of corticosteroid administration, respiratory distress syndrome is still at least twice as frequent as infection. In addition, intraventricular hemorrhage, the most crippling event resulting from prematurity, is primarily the result of the immaturity of the fetal brain and its fragile germinal matrix layer and hence its incidence would be reduced by extending intrauterine life through an expectant management approach.

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# Family trait analysis: A case-control study of 43 women with endometriosis and their best friends

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Analyses of lineage patterns show that for 43 women with endometriosis who reported that other family members have the disease, the vast majority of these familial cases involve the maternal lineage (79% to 93%). Of these women 34.9% of their mothers and 21.2% of their sisters were affected. With use of these rates, expected rates for first-degree relatives in a general population of women with endometriosis were determined. These rates were 6.2% and 3.8%, respectively; in combination, these rates yield an overall risk of 4.9% for first-degree relatives. Prior studies estimated this overall risk to be 6.9%; however, z scores determined that these rates do not differ statistically. Rates for second-degree maternal relatives are reported for grandmothers and aunts (0.4% compared to 3.1%, respectively), thus expanding results of former studies. In combination, an overall risk of 1.9% is estimated for second-degree relatives, as measured. (AM J OBSTET GYNECOL 1986;154:596-601.)

**Key words:** Family history, genetics, prevalence rates

Why does one woman develop endometriosis and another does not? Is there a predisposition for this disease to run in families? The work of two groups of investigators,<sup>1,4</sup> extending over a decade, lends credence to these questions posed by Ranney. At least for a subset of the population with endometriosis, there appears to be a tendency for patients to have other relatives with the disease.

Etiologic theories abound, including the still-popular theory proposed by Sampson in the 1920s that endometrial implants result from retrograde menstrual flow. Other theories include mechanical transport, metastatic, and mutation theories, among others.<sup>5-6</sup> Recent investigations pursue the link of a possible autoimmune reaction.<sup>7-9</sup> The plethora of theories has led others to question whether, indeed, practitioners are not dealing with more than one causative factor.

The indication that there may be a familial or genetic trait in operation in the disease was investigated by Ranney and his nursing staff in about 1970. After he noted that several patients in his private practice were related to each other, a mailed questionnaire to clients supported this hypothesis. Calculations based on his data show that 22% of Ranney's patients had relatives who had had surgical operations for endometriosis.

Ranney's analysis combines near and distant female relatives. Referral patterns were not taken into account.

The approach taken by investigators at Baylor College of Medicine—Simpson, Elias, Malinak, and Buttram<sup>3,4</sup>—consisted of interviews with 123 women who had surgery during the years 1971 through 1978. First-degree relatives were defined as female offspring, mothers, and sisters. Prevalence rates were calculated for both the patients and their husbands' families. The latter group acted as control subjects. By definition, second-degree relatives encompassed maternal grandmothers, aunts, and nieces. Third-degree relatives were defined as female first cousins. Neither second- nor third-degree relatives were included in the studies cited above. Hereafter, for brevity, we refer to these investigations as the Baylor studies.

The purpose of this investigation was to compare rates for first-degree relatives in our female control group with those of Baylor's control subjects in order to establish similarity. Expansion of the Baylor studies conducted with a sample of all women with endometriosis will be accomplished by determining rates for second-degree relatives in our registry of women with the disease. Rates for second- and even third-degree relatives may exist; however, published rates have not been located. Finally this report seeks to determine precisely which relatives are reported to have endometriosis.

## Material and methods

Complex medical, surgical, and fertility histories supplied by members of the Endometriosis Association are stored in a data registry at the Medical College of Wisconsin. The original questionnaire devised by the As-

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**Table I.** Sociodemographic characteristics of 43 respondents with endometriosis and 43 friend control subjects

	<i>Family history-positive group</i>	<i>Control group</i>	<i>Significance*</i>
Average age (yr)	32.7	32.9	NS
College education (%)	67%	56%	NS
White (%)	100%	95%	NS
Married (%)	69%	74%	NS
Average length of relationship (yr)	9.47	7.09	NS
Median annual income	30,250	31,500	
Occurance of endometriosis (%)	100	16.3	—

\**t* test or  $\chi^2$  values (uncorrected).

sociation did not seek pedigree or family history information. Since the instrument's revision, 485 of 491 women who were sent the revised version have responded to a general question: Do any of your blood relatives have endometriosis? Prior analyses showed that 18% ( $n = 87$  respondents) reported relatives of all degrees afflicted with the disease. Additional information supplied by respondents indicated that both paternal and maternal lineages were included in their responses; some volunteered information on suspected cases in their families.

To further define this familial linkage, the addresses of 83 of these 87 women were located and two identical questionnaires were mailed to each. The research design is similar to the "friend" methodologies described by the Centers for Disease Control and other state health departments during epidemiological investigations.<sup>10-13</sup> In the current study the respondents were requested to give the second questionnaire to any female good friend, excluding other members of the Endometrial Association or blood relatives. To increase the response rate, two mailings of questionnaires were sent 1 month apart. A total of two of 83 women in the registry had moved and were lost to follow-up.

One month after the second mailing a total of 66 subject responses had been received, for a total response rate of 82%, and 43 (65.2%) of their friends' questionnaires had been returned. These 43 sets of questionnaires formed the basis of the subject-control groups to be studied.

Both the Baylor control group and the control subjects in our study are envisioned to be representative of general populations of women. The female control group used by us, however, can be viewed as more representative of a population of women in their prime reproductive years, since no statistical differences in ages and racial backgrounds appeared between these cases and their female friends. For clarification, rates for Baylor's cases may be deemed representative of a population of all women with endometriosis. By contrast, rates determined for our cases were rates determined for a sample of women selected because of reports of a positive family history of others with the

disease. For comparison with both cases and controls at Baylor, rates calculated for the family history-positive group were extrapolated to the general population of all women with endometriosis represented in the registry.

Several estimations were made. First, the total number of sisters and maternal aunts, for example, had to be derived with use of the mean number of type of relative per individual. A factor of 2.02 was used to estimate these frequencies in the family history-positive group if in fact a 100% response rate to the questionnaire had been achieved. These figures were then extrapolated to the general population of women with endometriosis as represented in the registry. Finally, rates were compared with use of *z* scores. These rates were evaluated against rates reported by the Baylor investigators by the use again of difference of proportions tests.

Further questions were raised: How near in the family tree are these relationships? Are they maternally or paternally related? To clarify clients' original responses regarding blood relatives with the disease and to simultaneously gather information from a control group, both subjects and controls responded to an open-ended question: Do any members of your family have endometriosis? Respondents were asked to specify whom. Maternal and paternal relatives were listed. A series of questions then focused on the maternal lineage. Separate questions were asked about maternal grandmothers, the respondent's mother, her maternal aunts, her own sisters, and her own daughters.

Because the great majority of cases and controls in this study are young women, 16 of their 22 female offspring have not reached menarche and are not theoretically subject to the disease. The Baylor studies did not include offspring either, perhaps for this same reason.

## Results

How representative of the population, as represented by all 485 respondents, were the 87 subjects selected because of a positive family history? Analyses of five sociodemographic variables, age, marital

**Table II.** Numbers and percentages of first- and second-degree relatives, by type, and reporting relatives with endometriosis

	Family history—positive group (n = 43)		Friend control group (n = 43)	
	n	%	n	%
First-degree relatives				
Mothers				
No. of mothers	43		43	
Mothers with endometriosis	15	34.9	2	4.7
Sisters				
Patients with no sisters	15	34.9	14	32.6
Patients with sisters	28	65.1	29	67.4
Total No. of sisters	52		55	
Patients with sisters with endometriosis	10		1	
Sisters with endometriosis	11	21.2	1	1.8
Daughters				
Patients with daughters	8	18.6	14	32.6
Daughters with endometriosis	0*		0†	
Second-degree relatives				
Maternal grandmothers				
No. of grandmothers	43		43	
Grandmothers with endometriosis	1	2.3	1	2.3
Maternal aunts				
Patients with no aunts	10	23.3	10	24.4
Patients with aunts	33	76.7	31	75.6
Total No. of aunts	67		68	
No. of aunts with endometriosis	6		1	
Aunts with endometriosis	9	13.4	1	1.5

\*Four were &lt;12 years of age and four were ages 12 to 20.

†Twelve daughters were &lt;12 years of age; two were mature women.

status, education, income, and racial background, indicated no statistically significant differences on any variable. Later, family structure variables were examined to establish the representativeness of the 485 registry respondents to other published data. No differences in family structure were observed on these variables either.

How representative were the 43 women who furnished a friend control compared to the 44 who did not? The same five characteristics were again analyzed and no statistically significant differences were found by means of *t* tests of  $\chi^2$  examinations.

Of the 43 cases reporting a positive family history of others with the disease, only three cases (7.0%) involved the paternal lineage; 34 cases (79.1%) reported disease in the maternal lineage. An additional six cases (14.0%) reported relatives in the maternal lineage whose diagnoses were not firmly established as endometriosis.

Comparisons of the sociodemographic attributes of cases with characteristics of controls showed no statistical differences between groups (see Table I). The average age of cases and controls at the time of response was 33 years. A *t* test on means was not statistically significant. All 43 cases were white; 95% of the control group was white, not statistically different.

A total of 67% (n = 29) of the subject population were college graduates; 56% (n = 24) of the controls

were. A  $\chi^2$  test indicated no statistically significant differences. Sixty-nine and seventy-four percent of each group, respectively, were married although some of the others had current sexual partners. No statistical differences were observed between groups in the length of sexual relationships, as measured by a *t* test. Although a considerable number of each group did not provide information about their financial incomes, with this caveat in mind, a *t* test showed no differences in family incomes between clients and controls. All registry respondents reported firm diagnoses of endometriosis by means of laparoscopy or laparotomy; seven cases, or 16.3%, of the controls also reported having endometriosis.

**First-degree relatives.** Data in Table II are arrayed by types of first- and second-degree maternal lineages, as defined. These data are presented to show the percentages of each type of relative reported in the family history—positive and the friend-control groups and form the basis of rates reported in Table III.

Respondents' mothers, female siblings, and offspring are defined as first-degree relatives. Results in Table III show that we can expect 6.2% of mothers of all endometriosis clients in the registry to have endometriosis themselves. The comparable rate for the Baylor population was 8.1%. A difference of proportions test shows these percentages of mothers with endometriosis

**Table III.** Summary of expected prevalence risks for first- and second-degree relatives among women with endometriosis and among two control groups

	<i>Women with endometriosis</i>			<i>Control groups</i>	
	<i>Registry members (n = 485) (%)</i>	<i>Baylor patients (n = 123) (%)</i>	<i>Family history— positive group (n = 43) (%)</i>	<i>Families of Baylor patients' husbands (%)</i>	<i>Friend controls (n = 43) (%)</i>
First-degree relatives					
Mothers	6.2	8.1	34.9	0.9*	4.6
Sisters	3.8	5.8	21.2	1.0†	1.8
Second-degree relatives					
Maternal grandmothers	0.4	—	2.3	—	2.3
Maternal aunts	3.1	—	13.4	—	1.5

\*1:107 mothers.

†1:104 sisters.

to be comparable ( $z = 0.76$ ). This same examination compared the prevalence of mothers with endometriosis in Baylor's control group with mothers with the disease among friend controls. Again, no statistically significant differences were observed ( $z = 1.61$ ).

Data presented in Table III also show that 34.9% of mothers in the family history-positive group had endometriosis. Comparable percentages in each group had sisters among their siblings (65.1% compared to 67.4%, respectively), and the total number of sisters was similar. Twenty-one percent of the sisters in the family history-positive group had endometriosis.

Baylor's study found an endometriosis prevalence rate of 5.8% among patients' sisters. Our comparable estimated prevalence for sisters among all registry respondents was slightly lower, 3.8%, not statistically different from Baylor's results ( $z = 0.99$ ). We compared Baylor's rates for husbands' sisters with our female friend-control group. These rates were 1.0% compared to 1.8%, respectively. A  $z$  score of 0.50 showed no statistical difference among prevalences.

As was discussed earlier, few female offspring (total of 22) were reported by either subjects or controls. Only six daughters were menarcheal, defined as 12 years of age or older. No case of endometriosis was reported for any menarcheal offspring.

**Second-degree relatives.** Second-degree relatives encompass respondents' maternal grandmothers, maternal aunts, and siblings' offspring, or nieces. The questionnaire did not seek data on the total number of nieces; however, none were reported to have the disease in the open-ended question.

Data in Table II show that only one respondent in each group reported having a maternal grandmother with endometriosis. A rate of 2.3% was calculated for each group. As in the previous case with sisters, the percentage in each group reporting maternal aunts among their kinship ties was remarkably similar; 76.7% of the family history-positive group and 75.6% of the

controls had maternal aunts. The total numbers of aunts was almost identical; however, the percentages with endometriosis varied markedly. Rates determined for maternal grandmothers and aunts for the entire registry of women, with and without the family trait, were 0.4% and 3.1%, respectively. These estimates enlarged the work at Baylor to include second-degree relatives.

Of tangential interest were four cases reporting that the maternal grandmothers were suspected to have had endometriosis, although a diagnosis had never been accomplished. One of the controls reported a grandmother with endometriosis, but no other suspected cases were reported. A Fisher's exact test between suspected and definite cases among maternal grandmothers was not statistically significant ( $p = 0.33$ ).

### Comment

Ranney, Simpson, Malinak, Buttram, and Elias are among the leaders who have questioned the role of familial tendencies in endometriosis. The work done at the Baylor College of Medicine has contributed evidence strongly in support of a familial link, although the precise genetic patterns remain obscure. Moreover, the type of genetic determination is beyond the scope of this paper.

Our early analyses showed 18% of registry respondents reported other blood relatives were confirmed or suspected to have endometriosis. Calculations based on Ranney's data showed that 22% of his patients with endometriosis reported other relatives had had surgical operations for the condition. The 22% rate combines first- and second-degree relatives and perhaps even third-degree relatives. In addition, we were not able to tell with certainty that all second-degree relatives were maternally related. The data presented by the group at Baylor were limited primarily to a discussion of first-degree relatives only.

Results published by the Baylor researchers, for first-



degree relatives only, showed that 5.8% of their subjects' female siblings and 8.1% of their mothers were affected with endometriosis. For the husbands' families, only 1% of the husbands' female siblings and 0.9% of their mothers were so affected. For patients' families, an overall "recurrence risk" for all first-degree relatives was reported to be 6.9%. Daughters apparently were excluded in this analysis of first-degree relatives.

Our first task was to determine the similarity between our control group and the group used at Baylor. One percent of husband's sisters had endometriosis; our female friend-control group reported a similar figure of 1.8% (see Table II). Although only a 0.9 prevalence for husbands' mothers was found, 4.7% of the female friend-controls' mothers were reported to have endometriosis. A *z* score on the difference in proportions with endometriosis indicated no statistically significant difference in these two rates.

An overall prevalence risk for first-degree relatives of husbands' families per se was not reported; however, the authors at Baylor note that only two cases of endometriosis were reported for 211 husbands' siblings and mothers combined. With use of these data, the comparable overall prevalence risk for first-degree relatives in the husband-control sample was calculated to be 0.9%. The comparable rate for our female peer-control group for first-degree relatives was somewhat higher: 2.0%. The rate for second-degree relatives in our control group was 1.8%, still relatively small. No published data were located for comparison.

Finally, we have focused on the types of relatives with endometriosis. Recall that this study group is not representative of all women with endometriosis, but rather of women with endometriosis who also carry a positive family history. In fact, no study has emerged that truly is representative, since most are limited to readily available populations: hospital admissions, surgeries, or private practices, as Houston<sup>14</sup> has noted in an extensive review article. Using rates derived from our current study and applying these rates to all women seeking support services from the Endometriosis Association, we established rates for selected first- and second-degree relatives.

Malinak et al.<sup>4</sup> have remarked that "Patients with familial endometriosis represent an important population to study for clues to the possible origins of endometriosis . . ." Simpson furthers this encouragement. Although, as Simpson et al.<sup>3</sup> noted, their study did not investigate the likelihood that offspring of affected individuals also would be affected; they do remark, however, that "one could expect the risk to all first-degree relatives (sibs, parents, and offspring) to be similar because the percentage of shared genes between each is 50% . . . More distant relatives [not investigated by the Baylor team] (e.g., cousins, nieces) would be expected to be affected much less often, perhaps 1% or

less." Our results support these expectations. We established a certain prevalence for mothers and a lesser percentage for female siblings. Relatives further removed showed even lower rates.

Simpson et al. have well elaborated the practical considerations of a probable familial component. Included among these considerations are the counseling of patients regarding the risks held for subsequent generations, choices of contraceptives, and timing of childbearing. Certainly, knowledge leading to the identification of women and girls at risk of developing endometriosis holds implications for earlier diagnosis and intervention.

The question of whether the incidence of endometriosis is increasing is often raised. Changes in women's life-styles and delays in procreation are variables commonly thought to influence these possibly increasing rates. More sophisticated diagnostic, laboratory, and surgical interventions, as well as the dissemination and accessibility of medical care, also contribute to increases in rates.

It is difficult, among myriad predisposing factors such as these, to single out an inheritability factor. Indeed, several practitioners have questioned whether we are not dealing with a disease of multiple etiologies. Pooling data may well obscure definite, and distinct, risk factors in operation. Yet the patterns that continue to emerge imply that, at least for a subpopulation of women with endometriosis, a familial trait does exist. And, as the researchers at Baylor have reported, this subpopulation suffers more serious disease. Supporting this finding, our own analyses, not reported here, have shown that for these women the symptoms present earlier in life and greater disability is sustained. Whether this observed familial trait is truly genetic in origin or is related to shared life-styles and behaviors among family members remains to be seen.

We noted and were puzzled by the relatively few maternal grandmothers reported as having the disease. Whether this reflects the possible dearth of endometriosis-oriented diagnostic methods available to this generation or real changes in operation affecting subsequent and more contemporary mother-daughter pairs cannot be determined.

The causes and the cures for this often serious disease remain elusive. However, physicians are now armed with more sophisticated diagnostic and surgical tools; genetic and biological laboratory techniques are now available. Longitudinal studies involving this current generation and their own daughters may well clarify this perplexity.

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## $\alpha$ -Adrenergic receptors in human myometrium during pregnancy

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The distribution and mechanisms of  $\alpha$ -adrenergic receptors have been studied in myometrial preparations from women delivered by cesarean section at term. With the use of the radioligand tritiated dihydroergocryptine, the number of  $\alpha$ -adrenergic receptors was 210 fmol/mg of protein in the uterine fundus and 195 fmol/mg of protein in the lower uterine segment. Competition experiments showed that 60% of the  $\alpha$ -adrenergic receptors had properties as  $\alpha_1$ -adrenergic receptors and 40% as  $\alpha_2$ -adrenergic receptors. In vitro tension studies verified the existence of physiologically active  $\alpha_1$ - and  $\alpha_2$ -adrenergic receptors in the myometrial preparations.  $\alpha_2$ -Adrenergic receptor stimulation resulted in lowered levels of intracellular cyclic adenosine monophosphate. The intracellular cyclic adenosine monophosphate was further reduced by additional  $\alpha_1$ -adrenergic receptor stimulation, probably secondary to an activation of calcium-calmodulin-dependent phosphodiesterase. (AM J OBSTET GYNECOL 1986;154:601-6.)

**Key words:**  $\alpha$ -Adrenergic receptors, myometrium, labor, cyclic adenosine monophosphate

There is considerable experimental evidence that the human myometrium contains both  $\beta$ - and  $\alpha$ -adrenergic receptors.<sup>1,2</sup> The mechanism of action of  $\beta$ -adrenergic receptors in the myometrium has been thoroughly studied.<sup>3</sup> However, the distribution of  $\alpha$ -adrenergic receptors in human myometrium has just recently been investigated and only in nonpregnant women.<sup>2</sup> The

aim of this study was, therefore, to evaluate the  $\alpha$ -adrenergic receptor distribution and function in myometrium from pregnant women and to study in a conventional in vitro system the effect of different pharmacologic  $\alpha$ -adrenergic receptor blockers on spontaneously contracting myometrium and in receptor-binding assays in order to classify the subtypes of  $\alpha$ -adrenergic receptors and to look for possible new means to improve today's tocolytic treatment.

### Material and methods

**Patients.** Myometrial biopsy specimens were taken during cesarean section from the lower uterine segment and/or the uterine fundus. The reason for cesarean section was related to obstetrics in all cases. The gestational ages were between 37 and 41 weeks and

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active labor had not started in any patients at the time of operative delivery. About two thirds of the women were anesthetized with an epidural block, and the remainder received the conventional intravenous anesthetics thiopental sodium and leptanale. The myometrial preparations were immediately immersed in Ringer's solution and transported to the laboratory. Biopsy specimens from a total of 48 women were studied.

**Studies of contractility in vitro.** The myometrial preparations were mounted in special holders for registration of isometric tension by FTO3 transducers on a Grass polygraph. The myometrial strips were suspended in a Krebs buffer solution at 37° C with the following composition: sodium chloride, 118.8 mmol/L; potassium chloride, 4.7 mmol/L; calcium chloride, 2.2 mmol/L; magnesium chloride, 1.2 mmol/L; sodium bicarbonate, 23.8 mmol/L; potassium phosphate, 1.2 mmol/L; glucose, 5.5 mmol/L. The solution was aerated with a gas mixture containing 95% oxygen and 5% carbon dioxide. All myometrial preparations were either untreated or pretreated with (1) phentolamine ( $\alpha_1$ - and  $\alpha_2$ -adrenergic receptor blocker), (2) yohimbine ( $\alpha_2$ -adrenergic receptor blocker), and (3) prazosin ( $\alpha_1$ -adrenergic receptor blocker). All preparations were then treated with increasing concentrations of norepinephrine and the tension was measured.

**Preparation of myometrial membranes.** The myometrial tissue was cut into small pieces and homogenized in 10 volumes of buffer solution (sucrose, 0.25 mol/L; Tris, 5 mmol/L; magnesium chloride, 1 mmol/L; pH 7.4) with the use of a Polytron PT 10. During the preparation procedure, the myometrial tissue was kept in ice-cold buffer solution. The homogenate was filtered through a cheese-cloth and thereafter centrifugated at 400  $\times$  g for 10 minutes at 4° C. The supernatant was centrifugated at 28,000  $\times$  g for 10 minutes. The resulting pellets were washed and recentrifuged twice at 28,000  $\times$  g. The washed pellets were suspended in three volumes of incubation buffer (Tris, 50 mmol/L; magnesium chloride, 10 mmol/L; pH 7.5) and used for the determination of tritiated dihydroergocryptine binding.

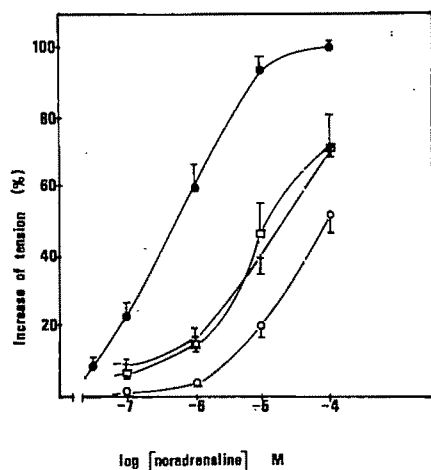
**Binding of tritiated dihydroergocryptine.** Binding of tritiated dihydroergocryptine to human myometrial membranes was assayed by filtration of the preparations on glass fiber filters.<sup>7</sup> Membrane proteins were incubated with the indicated concentrations of tritiated dihydroergocryptine and incubation buffer in a total volume of 0.15 ml for 20 minutes at 37° C. At the end of incubation the samples were diluted in 2 ml of ice-cold incubation buffer, followed by rapid vacuum filtration through Whatman glass fiber filters on a Millipore Sampling Manifold. The filters were then rapidly washed six times with 3 ml of the ice-cold in-

cubation buffer. After drying, radioactivity bound to the filters was determined on a Packard liquid scintillation spectrometer. Specific binding was determined by subtracting nonspecific binding from total binding. In all figures and tables, bound tritiated dihydroergocryptine refers to specific binding. Nonspecific binding was determined in the presence of phentolamine, 10  $\mu$ mol/L. Protein was determined according to Lowry et al.<sup>4</sup>

**Determination of cyclic adenosine monophosphate in myometrial tissue.** Stimulation with norepinephrine (1  $\mu$ mol/L) of the myometrial strip was performed in an organ bath with or without prazosin (1  $\mu$ mol/L) or yohimbine (1  $\mu$ mol/L) and in the same manner with myometrial preparations pretreated with propranolol (1  $\mu$ mol/L). After treatment with norepinephrine for 3 minutes in the organ bath, the strips were dismantled and frozen in Frigen 12 and solid carbon dioxide at -70° C.

The frozen preparations were homogenized in perchloric acid and the nucleotides were separated on columns of Ag-1-X-8 (200 to 400 mesh, formate form). After elution with formic acid the eluates were lyophilized. The cyclic adenosine monophosphate content was determined according to Gilman.<sup>5</sup>

**Determination of phosphodiesterase activity.** The activity of phosphodiesterase was determined by the method described by Thompson and Appleman<sup>6</sup> with minor modifications. After 30,000  $\times$  g centrifugation of the myometrial homogenate for 10 minutes, the supernatant was used for analysis. The assay was performed in a solution containing Tris hydrochloride, 40 mmol/L, pH 7.5; magnesium chloride, 5 mmol/L; 20  $\mu$ g of 5-nucleotidase (snake venom) and tritiated cyclic adenosine monophosphate, 1  $\mu$ mol/L, containing more than 100,000 cpm, in a total volume of 0.1 ml. After 3 to 5 minutes at 37° C the reaction was terminated by heating the reaction mixture at 90° C for 1 minute, and 0.4 ml of distilled water was then added to the incubation tube. The entire contents of the tubes were transferred to prewashed AG1  $\times$  8 columns (C1 form, 0.5 by 1.0 cm) followed by addition of 1 ml of distilled water for sampling of fractions containing the tritiated adenosine. The activity was measured in a liquid scintillation counter. Calcium-dependent phosphodiesterase activity was measured in six strips. Ionized calcium was added in increasing concentrations to an enzyme preparation dialyzed in Tris buffer containing ethyleneglycol-bis( $\beta$ -amino-ethyl ether)triacetic acid (EGTA), and phosphodiesterase activity was measured as described above. In 12 myometrial preparations trifluoperazine was added in increasing concentrations (0.1  $\mu$ mol/L to 1 mmol/L of trifluoperazine) (see Fig. 5, A and B).



**Fig. 1.** Tests of antagonists on the contractile effects of norepinephrine (noradrenaline) in human myometrial preparations pretreated with propranolol ( $10^{-6}$  mol/L). In the tests of the antagonists, the myometrium was preincubated with prazosin ( $10^{-6}$  mol/L) ( $\square$ ), yohimbine ( $10^{-6}$  mol/L) (+), or phentolamine ( $10^{-6}$  mol/L) ( $\circ$ ) for 10 minutes before the norepinephrine tests were performed. Values are means  $\pm$  SEM.

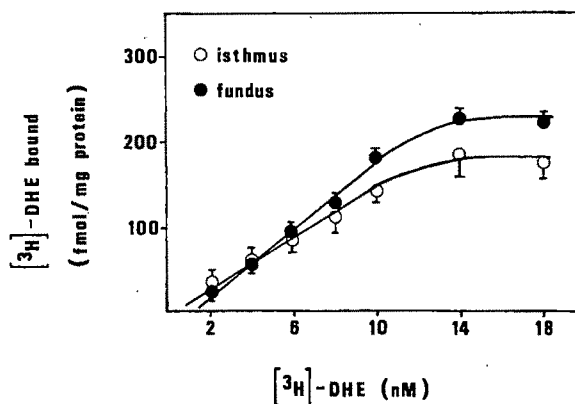
**Pharmacologic agents.** Tritiated dihydro- $\alpha$ -ergocryptine (50 Ci/mmol) (New England Nuclear, Dreieich, West Germany), L-norepinephrine bitartrate (Sigma Chemical Co., St. Louis, Missouri), phentolamine methansulfon (Ciba Geigy AG, Basel, Switzerland), prazosin hydrochloride (Pfizer, Inc., New York, New York), DL-propranolol hydrochloride (ICI, Macclesfield, Cheshire, England), trifluoperazine dihydrochloride (Sigma Chemical Co.), yohimbine hydrochloride (Sigma Chemical Co.).

**Statistical methods.** All values are given as mean values  $\pm$  SEM. Levels of significance were tested by Student's *t* test with the use of paired and unpaired data. The study was approved by the Ethical Committee, Linköping University.

## Results

**Contractility.** Norepinephrine in a concentration of  $10^{-7}$  to  $10^{-4}$  mol/L increased the tension in a dose-dependent manner in spontaneously contracting myometrial strips from the fundus uteri as well as from the lower uterine segment (Fig. 1). The  $EC_{50}$  of the contractile effect of norepinephrine was  $5 \times 10^{-7}$  mol/L. The dose-effect curve was markedly shifted to the right when the strips were pretreated with phentolamine,  $1 \mu\text{mol/L}$  (Fig. 1). The  $EC_{50}$  value in the presence of phentolamine was about  $10^{-4}$  mol/L.

When the myometrial strips were pretreated with either prazosin,  $1 \mu\text{mol/L}$ , or yohimbine,  $1 \mu\text{mol/L}$ , before norepinephrine stimulation, a shift to the right of the dose-response curve was registered in both groups. The  $EC_{50}$  (the concentration that increases the tension



**Fig. 2.** Number of  $\alpha$ -adrenergic receptor binding sites in the lower uterine segment (isthmus) and uterine fundus of untreated pregnant women. Values are means  $\pm$  SEM.

50%) values were  $1.7 \times 10^{-5}$  and  $2.5 \times 10^{-5}$  mol/L in the presence of prazosin and yohimbine, respectively, indicating the presence of physiologically active  $\alpha_1$ - and  $\alpha_2$ -adrenergic receptors in the myometrium of pregnancy.

**Tritiated dihydroergocryptine binding.** Binding of tritiated dihydroergocryptine to human myometrial membrane associated with pregnancy is shown in Fig. 2, where data represent specific binding (total minus nonspecific binding). Nonspecific binding was measured in the presence of phentolamine,  $10 \mu\text{mol/L}$ . The total numbers of specific binding sites were about 195 and 210 fmol/mg of protein in the lower uterine segment and uterine fundus, respectively (Fig. 2). The affinity of the receptors was comparable in the two locations as the equilibrium dissociation constants ( $K_d$ ) of tritiated dihydroergocryptine were almost equal, 6 and 6.2 nmol/L, respectively.  $\alpha$ -Adrenergic receptor antagonists potently competed for the binding sites. The competitive antagonist phentolamine competed with a  $K_d$  of 28 nmol/L (Fig. 3, A). Competition experiments with the  $\alpha_1$ -adrenergic receptor antagonist prazosin and the  $\alpha_2$ -adrenergic receptor antagonist yohimbine (Fig. 3, B) gave clear support for the notion that tritiated dihydroergocryptine bound to two sites. The estimated  $K_d$  value was 0.5 nmol/L for the high-affinity site of prazosin and 1 nmol/L for yohimbine. The proportions of  $\alpha_1$ - and  $\alpha_2$ -adrenergic receptor sites were 60% and 40%, respectively, of the total tritiated dihydroergocryptine binding capacity.

**Cyclic adenosine monophosphate.** In both untreated and propranolol-pretreated myometrial biopsy specimens, cyclic adenosine monophosphate production was significantly reduced when stimulated with norepinephrine,  $1 \mu\text{mol/L}$  (Fig. 4, A and B). Prazosin and yohimbine partly inhibited the norepinephrine-induced reduction of the cyclic adenosine mono-



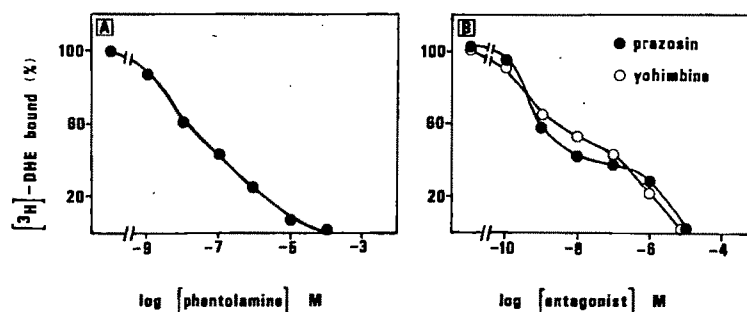


Fig. 3. Inhibition of tritiated dihydroergocryptine ( $[^3H]$ -DHE) binding to  $\alpha$ -adrenergic receptors by  $\alpha$ -adrenergic receptor antagonists. A: Phentolamine. B: Prazosin and yohimbine.

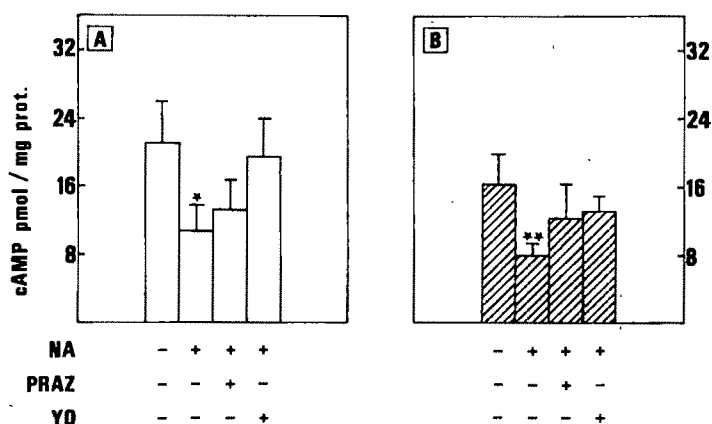


Fig. 4. Effects of norepinephrine (NA) ( $10^{-6}$  mol/L) on the cyclic adenosine monophosphate (cAMP) levels of human myometrium in the absence or presence of prazosin (PRAZ) ( $10^{-6}$  mol/L) or yohimbine (YO) ( $10^{-6}$  mol/L). A: Preincubation in normal Krebs buffer. B: Preincubation in Krebs buffer containing propranolol,  $10^{-6}$  mol/L. Values are means  $\pm$  SEM. The significance of the changes from control values are indicated by \* =  $p < 0.05$  and \*\* =  $p < 0.01$ .

phosphate content in the propranolol-pretreated preparations (Fig. 4). In the absence of propranolol, yohimbine almost completely reversed the norepinephrine response while prazosin had no significant effect (Fig. 4, A).

**Phosphodiesterase.** The lowering effect on cyclic adenosine monophosphate seems to be mainly due to an activation of  $\alpha_2$ -adrenergic receptors, while  $\alpha_1$ -adrenergic receptors were involved to a minor extent. Changes in ionized calcium are suggested to play an important role in the  $\alpha_1$ -adrenergic receptor response.<sup>8</sup> Calcium ions also influence the cyclic adenosine monophosphate-phosphodiesterase activity, at least in the brain.<sup>9</sup> We therefore studied the influence of ionized calcium ions on the phosphodiesterase activity obtained from human myometrium and found that increasing concentrations of calcium ( $10^{-6}$  to  $10^{-3}$  mol/L) caused a slight but significant increase in phosphodiesterase activity (Fig. 5, A). When the calmodulin-inhibitory drug trifluoperazine was tested, a remarkable inhibition of the phosphodiesterase activity was noticed in the concentration interval  $10^{-6}$  to  $10^{-3}$  mol/L (Fig. 5, B).

## Comment

In the present study we have investigated  $\alpha$ -adrenergic receptors of the human myometrium in pregnancy with two conventional in vitro systems, tension recordings and receptor-binding assays.

In the tension studies, both  $\alpha_1$ - and  $\alpha_2$ -adrenergic receptors seemed to be involved in the smooth muscle contraction mechanism. The  $\alpha_1$ - and  $\alpha_2$ -adrenergic receptor blocking agent phentolamine was approximately doubly effective in preventing increased tension after norepinephrine stimulation when compared with either the  $\alpha_1$ -blocker prazosin or the  $\alpha_2$ -blocker yohimbine (Fig. 1).

In the receptor-binding assays we chose the radioligand tritiated dihydroergocryptine and discriminated  $\alpha_1$ - and  $\alpha_2$ -adrenergic receptors by studying the displacement of tritiated dihydroergocryptine by selective  $\alpha$ -antagonists as described by Hoffman et al.<sup>10</sup> Our results indicate that both  $\alpha_1$ - and  $\alpha_2$ -adrenergic receptors are present in myometrium from pregnant women in the proportion 60:40 and that the distribution of  $\alpha$ -adrenergic receptors is comparable in the lower uterine

segment and uterine fundus (Fig. 2). Wikland et al.<sup>11</sup> recently demonstrated a marked reduction in fluorescent nerve fibers in myometrium at term compared with myometrium from nonpregnant women. They concluded that neuronal factors of maternal origin are unlikely to be involved in the myometrial contractile response in pregnant uteri at term. The  $\alpha_1$ - and  $\alpha_2$ -adrenergic receptors demonstrated in our study are probably localized on the myometrial cells, since very few nerve fibers were present in term myometrium. Both  $\alpha$ -adrenergic receptor subtypes seem to be of importance for eliciting the excitatory muscle response. The mechanisms believed to mediate the responses of the two receptors seem, however, to differ. Previous studies on  $\alpha_2$ -adrenergic receptors in platelets, for instance, have shown that stimulation of  $\alpha_2$ -adrenergic receptors lowers the intracellular cyclic adenosine monophosphate, probably through an inhibition of adenylate cyclase.<sup>8</sup> The same mechanism seems to be applicable in human myometrium in pregnancy (Fig. 4, A and B). However, an additional effect on the cyclic adenosine monophosphate metabolism was seen after stimulation of  $\alpha_1$ -adrenergic receptors, especially when the myometrial biopsy specimens were pretreated with a  $\beta$ -blocker, propranolol. One explanation of this could be that  $\alpha_1$ -adrenergic receptor stimulation alters the intracellular ionized calcium content, as found in liver cells by Exton<sup>8</sup> and in fatty cells by Garcia Sainz,<sup>12</sup> and thus secondarily reduces cyclic adenosine monophosphate. In the present study we were able to show that an increase in ionized calcium caused a small but significant increase in cyclic adenosine monophosphate-dependent phosphodiesterase, which might in turn reduce the cyclic adenosine monophosphate content. The calmodulin antagonist trifluoperazine reduced phosphodiesterase activity in a dose-dependent manner (Fig. 5, A and B), indicating the presence of a calcium-calmodulin-dependent phosphodiesterase. This has previously been demonstrated in the brain.<sup>9</sup>

Recent publications concerning preterm labor and  $\beta$ -sympathomimetic treatment have suggested a role of endogenous  $\alpha$ -adrenergic stimulation in explaining the often limited effect of  $\beta$ -sympathomimetics. In a study on human myometrium in pregnancy by Ke et al.,<sup>13</sup>  $\beta$ -sympathomimetics were administered intermittently to the *in vitro* preparations and in this way the investigators could avoid down-regulation of  $\beta$ -adrenergic receptors. They also postulated that in clinical practice patients treated with  $\beta$ -sympathomimetics showed a higher endogenous catecholamine level, which might stimulate the myometrium to contractions, at least when the  $\beta$ -adrenergic receptor system is down-regulated, as has been demonstrated after terbutaline treatment.<sup>1</sup>

The clinical importance of the  $\alpha$ -adrenergic receptor

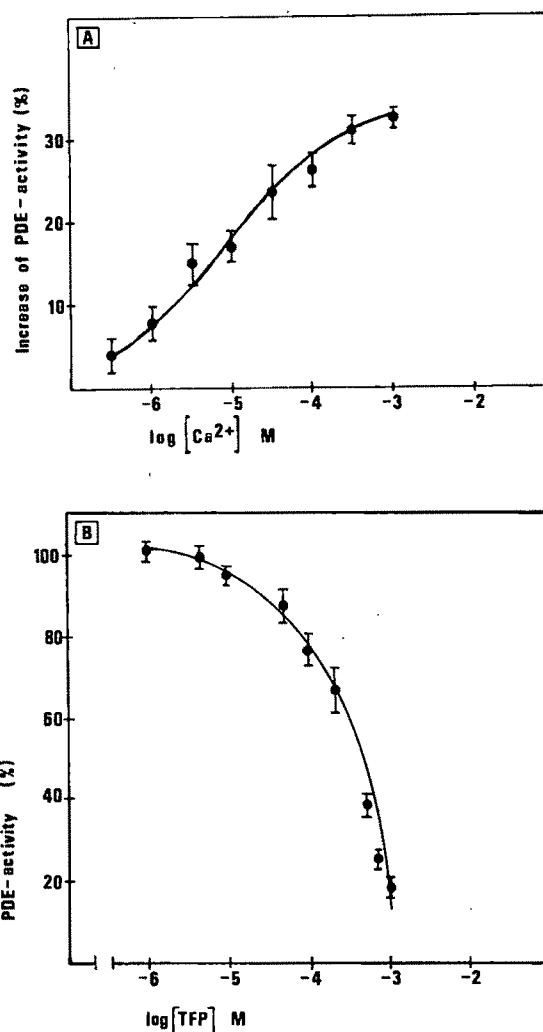


Fig. 5. A: The effect of calcium on human myometrial phosphodiesterase activity. The enzyme preparation was dialyzed against Tris buffer containing 1 mmol/L of EGTA before the assay. Values are means  $\pm$  SEM. B: The effect of different concentrations of trifluoperazine on cyclic adenosine monophosphate-phosphodiesterase (PDE) activity. Values are means  $\pm$  SEM.

system cannot be stated before further studies on the number of  $\alpha$ -adrenergic receptors in preterm and term pregnancies with and without labor have been performed.

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## Corticotropin-releasing factor can stimulate gonadotropin secretion by human fetal pituitaries in superfusion

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In previous studies, corticotropin-releasing factor was found to elicit a rise in circulating adrenocorticotrophic hormone in human subjects and laboratory animals, but no stimulatory effect of corticotropin-releasing factor on other pituitary hormones was detected. Since stress may be associated with luteinizing hormone changes as well as with those of corticotropin-releasing factor and adrenocorticotrophic hormone, we quantified gonadotropin responses to corticotropin-releasing factor and arginine vasopressin in 11 human fetal pituitaries with use of both superfusions and static incubations. Exposure to corticotropin-releasing factor brought about a significant increase in adrenocorticotrophic hormone and gonadotropin concentrations in the effluent media by means of the superfusion system. Similar concentrations of corticotropin-releasing factor significantly increased adrenocorticotrophic hormone secretion into the medium by dispersed fetal pituitary cells cultured on an extracellular matrix but failed to increase luteinizing hormone and follicle-stimulating hormone secretion. Exposure to 3 mmol/L 8-bromo-cyclic adenosine monophosphate caused an increase in all three peptides, both in superfusion and static incubations. Dose-response studies showed that the effect on gonadotropin secretion occurred at concentrations of 8-bromo-cyclic adenosine monophosphate two orders of magnitude lower than those affecting adrenocorticotrophic hormone secretion. The purity of corticotropin-releasing factor and arginine vasopressin used in these studies was confirmed by high-performance liquid chromatography. These *in vitro* results are consistent with a paracrine effect of corticotropes acting on gonadotropes. The combination of static incubation and superfusion has proved useful in elucidating the effects of different secretagogues on pituitary cells. (*AM J OBSTET GYNECOL* 1986;154:606-12.)

**Key words:** Fetal pituitary, gonadotropin

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Shortly after the elucidation of the structure of corticotropin-releasing factor by Vale et al.,<sup>1</sup> there were several studies in which its effects on the secretion of adrenocorticotrophic hormone, growth hormone, thyroid-stimulating hormone, luteinizing hormone, and prolactin in laboratory animals<sup>2,3</sup> and in normal humans<sup>4,5</sup> were assessed. Whereas corticotropin-releasing factor was found to elicit a rise in circulating adreno-

corticotrophic hormone and subsequently in cortisol and aldosterone, no stimulatory effect of corticotropin-releasing factor on the other pituitary hormones was found in normal human subjects.<sup>3</sup> Similarly other investigators were unable to demonstrate a rise in plasma thyroid-stimulating hormone or luteinizing hormone in monkeys.<sup>3</sup>

However, since stress has been reported to stimulate<sup>6</sup> or inhibit<sup>7</sup> luteinizing hormone secretion in rats, and since adrenocorticotrophic hormone is secreted in response to stress<sup>8</sup> as well as to corticotropin-releasing factor,<sup>1-5</sup> we quantified gonadotropin responses to corticotropin-releasing factor in human fetal pituitaries in vitro. Studies were performed with use of both superfusion of pituitary fragments and static incubations of dispersed pituitary cells.

### Material and methods

Eleven human fetuses, 14 to 25 weeks' gestation, were obtained within 2 hours of therapeutic termination of pregnancy by evacuation after laminaria-induced dilatation. In the first six cases the fetal pituitary was removed, placed in ice-cold medium 199/Earle's basic salt solution, and transported to the laboratory. Each pituitary was divided into six fragments to increase surface area, which were placed in a tissue chamber containing superfusion medium and submerged in a 37° C water bath. The superfusion chamber was composed of an O-ring Pyrex joint (G9235-1/11, Corning 6780, SP, McGraw-Park, Illinois) with an internal diameter of 5 mm. The pituitary fragments were located on a 0.5 mm thick scintered glass disk filter fitted with a rubber gasket and held between the Pyrex joints by a metal clamp. The superfusion medium was medium 199/Earle's basic salt solution, prepared by the cell culture facility of the University of California, San Francisco, containing 0.1% bovine serum albumin (fraction V, 98% to 99% albumin, Sigma Chemical Co., St. Louis, Missouri),  $20 \times 10^{-6}$  mol/L of bacitracin,  $10^{-3}$  mol/L of ascorbate, 25 mmol/L of N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (Hepes buffer) and saturated with 95% oxygen and 5% carbon dioxide.

The superfusion system was modified from that described by Kraicer and Chow.<sup>9</sup> In brief, the medium was pumped from a 37° C reservoir by a Manostat (New York, New York) peristaltic pump, at a rate of 0.3 ml/min through a four-way valve (SRV-4, Pharmacia Fine Chemicals, Piscataway, New Jersey). This system permitted instant changing of the medium inflow into the chamber with minimal pressure changes. All connecting tubing was PE 160 (inside diameter, 0.045 inch; Clay Adams, Parsippany, New York), and the connectors were stainless steel (Small Parts Inc., Miami, Florida). The effluent from the tissue chamber was collected on ice in sequential 10-minute fractions with use of a frac-

tion collector (ISCO, Lincoln, Nebraska, Model 328). The effluent was divided into three aliquots and stored at -70° C until assay.

Synthetic human corticotropin-releasing factor was purchased from Peninsula Laboratories (San Carlos, California), dissolved in  $10^{-3}$  N HCl in normal saline solution, and slowly injected into the superfusion system (first three superfusions). In the next three superfusions the corticotropin-releasing factor was diluted in the superfusion medium within 5 minutes, equilibrated with oxygen/carbon dioxide in a reservoir identical to the medium reservoir, and administered as a 10-minute "square-wave" through the peristaltic pump with use of the four-way valve to minimize pressure changes.

Arginine vasopressin (Bachem, Torrance, California) was dissolved in normal saline solution (pH 4.5) and then diluted in medium. Potassium chloride was dissolved in superfusion medium at a concentration of 60 mmol/L and administered as a 10-minute square-wave at the end of each superfusion.

8-Bromo-cyclic adenosine monophosphate (cAMP) was obtained from Sigma Chemical Co.

### Preparation of dispersed cells and static incubations

**Materials.** Dulbecco's modified Eagle's medium H-16, medium 199/Earle's basic salt solution; Hanks calcium- and magnesium-free buffer, fetal bovine serum, and saline solution with 0.1% trypsin-versine were supplied by our cell culture facility. All medium contained 100 µg/ml of penicillin, 100 µg/ml of streptomycin, 2 µmol/L of glutamine, and 25 mmol/L of Hepes buffer. Fibroblast growth factor was purified from bovine brains according to the method of Gospodarowicz et al.<sup>10</sup> Tissue culture dishes were purchased from Falcon (Los Angeles, California). All cultures were maintained at 37° C in a Forma (Marietta, Ohio) CH/P incubator with 95% oxygen and 5% carbon dioxide.

**Extracellular matrix.** The use of an extracellular matrix derived from bovine corneal endothelial cells for maintenance of human pituitary cells in culture has been described previously from these laboratories.<sup>11</sup> The extracellular matrix derived from these cells has now been used extensively for the culture of various normal and tumor cells. Recently the use of this matrix provided a successful means for culturing human pituitary adenoma cells, which adhere poorly to plastic.<sup>11</sup> The use of extracellular matrix can also circumvent the need for various attachment factors in serum.<sup>12</sup> Culture of bovine corneal endothelial cells and the production of extracellular matrix were performed according to the method of Gospodarowicz et al.<sup>13</sup> In brief, for matrix production, stock cultures of corneal endothelium were split with trypsin-versine, and the corneal endothelial cells ( $3 \times 10^4$ /ml) were plated in 24 well plates with



Dulbecco's modified Eagle's medium containing 15% fetal bovine serum, 5% (wt/vol) of Dextran T-40, and antibiotics as described above. Fibroblast growth factor (100 ng/ml) was added three times during the first week. After reaching confluence (within 6 to 7 days), the cultures were incubated 7 to 10 additional days during which the matrix was deposited. The cells were removed with a rapid wash in 20 mmol/L of ammonium hydroxide. The matrix was rinsed twice with phosphate-buffered saline solution, pH 7.4, and the plates, containing phosphate-buffered saline solution and antibiotics, were stored at 4° C.

**Pituitary dispersion.** Each of the five fetal pituitaries used for dispersion for static incubations (ages 16 to 23 weeks' gestation) was removed within 2 hours of pregnancy termination and placed in Hanks calcium- and magnesium-free buffer at room temperature. The tissue was minced in fresh Hanks buffer, and the fragments were transferred to a 15 ml conical tube and allowed to settle. The buffer was aspirated, and 5 ml of Hanks calcium- and magnesium-free buffer containing 0.5% collagenase and 50 µg deoxyribonuclease were added. This was incubated at 37° C for 1 hour with agitation in a Dubnoff metabolic shaker, after which 50 µg of deoxyribonuclease were added and the tissue was mechanically dispersed in 5 ml of Hanks calcium- and magnesium-free buffer by trituration with a 5 ml pipette. Any remaining fibrous tissue fragments were allowed to settle and then removed. The cell suspension was centrifuged at  $150 \times g$  for 5 minutes, and the cell pellet was resuspended in medium 199/Earle's basic salt solution containing 10% fetal bovine serum and plated at a density of  $7.5$  to  $10 \times 10^4$  cells per extracellular matrix-coated well.

After 3 to 4 days in culture, the cells, which by this time were firmly attached to the extracellular matrix, were washed and preincubated with serum-free medium containing 0.1% bovine serum albumin. After a 90-minute preincubation period, the cells were washed again, and secretagogues were added in medium 199/Earle's basic salt solution containing 0.1% bovine serum albumin, penicillin/streptomycin, Hepes buffer, and glutamine, 1 ml per well. Each treatment was performed in quadruplicate or quintuplicate wells. After 3-hour incubation with either medium (control), 10 nmol/L of corticotropin-releasing factor, 100 nmol/L of arginine vasopressin, a combination of these secretagogues, or 8-bromo-cAMP, the medium was collected in cold polystyrene Eppendorf tubes, divided into aliquots, and kept at -70° C until assay.

The adrenocorticotrophic hormone radioimmunoassay<sup>14</sup> used rabbit antiadrenocorticotrophic hormone (1-24) serum, generously provided by Dr. S. Hane, Metabolic Research Unit, University of California, San Francisco. Adrenocorticotrophic hormone (1-39) (Cal-

biochem, La Jolla, California) was used for the standard curve. The adrenocorticotrophic hormone trace, supplied by Dr. Hane, was iodinated with use of a modification of the method of Hunter and Greenwood.<sup>15</sup>

After 48 hours' incubation of samples with antiserum and trace, separation of bound hormone from free hormone was accomplished by addition of goat antirabbit  $\gamma$ -globulin. The cross-reactivity with  $\alpha$ -melanocyte-stimulating hormone was less than 0.1%, and the intraassay and interassay variations were <7.5% and <10%, respectively. The assay sensitivity was 1 pg of adrenocorticotrophic hormone per tube. The sample volume was 20 to 100 µl per tube. The final antiserum concentration was 1:75,000. Gonadotropins were measured by radioimmunoassays similar to those previously described from this laboratory<sup>16</sup> using antisera kindly supplied by Dr. A. F. Parlow (antihuman luteinizing hormone AFPC65811) and the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases (antihuman follicle-stimulating hormone batch 3).

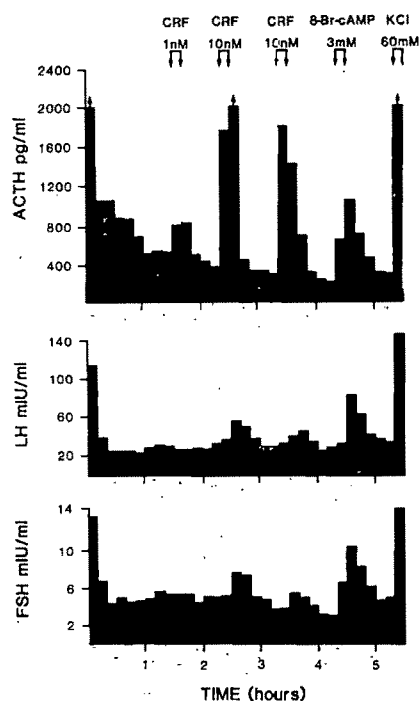
There was no detectable cross-reactivity with adrenocorticotrophic hormone in the follicle-stimulating hormone or luteinizing hormone assays. Luteinizing hormone and follicle-stimulating hormone concentrations were expressed in terms of milliinternational units of the second international reference preparation of human menopausal gonadotropin. The minimal concentrations of gonadotropin that could be quantified in these assays (90% binding) were 1 mIU/ml for follicle-stimulating hormone and 2.5 mIU/ml for luteinizing hormone.

The purity of corticotropin-releasing factor and arginine vasopressin was confirmed by high performance liquid chromatography. Corticotropin-releasing factor was chromatographed on a C-18 column with use of a flow rate of 1 ml/min, and a linear gradient over 40 minutes from 100% 0.05 mol/L of monosodium acid phosphate to 40% acetonitrile in the phosphate buffer. Arginine vasopressin was analyzed on a C-18 column by means of the method of O'Hare and Nice.<sup>17</sup>

Statistical comparisons were made by Student's *t* test and one-way analysis of variance (Newman-Keuls test).

## Results

The adrenocorticotrophic hormone, luteinizing hormone, and follicle-stimulating hormone secretory responses of pituitary fragments in a representative superfusion of a fetus of 18.1 weeks are shown in Fig. 1. Injection of vehicle alone did not cause any change in the concentrations of any of the measured pituitary peptides. Exposure to 1 nmol/L of corticotropin-releasing factor for 10 minutes caused a 35% increase in adrenocorticotrophic hormone above baseline but did not cause any significant luteinizing hormone or follicle-stimulating hormone change. However, exposure to

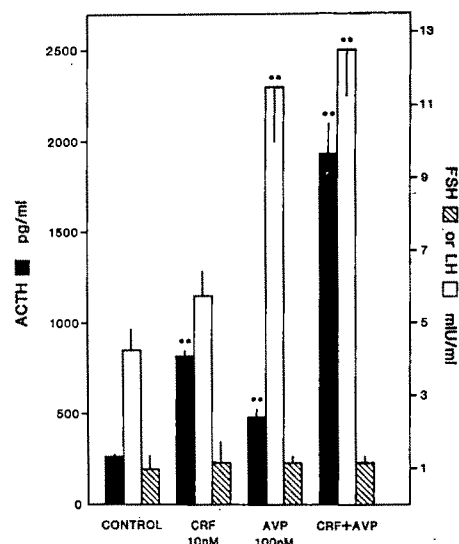


**Fig. 1.** Effects of corticotropin-releasing factor (CRF), 8-bromo-cAMP, and potassium chloride (KCl) on adrenocorticotrophic hormone (ACTH), luteinizing hormone (LH), and follicle-stimulating hormone (FSH) secretion by superfused human pituitary fragments in a representative superfusion of a fetus of 18.1 weeks.

10 nmol/L of corticotropin-releasing factor or more brought about a fourfold to fivefold increase in the concentration of adrenocorticotrophic hormone ( $n = 6$ ) and a 1.5- to twofold increase in luteinizing hormone ( $n = 6$ ) and follicle-stimulating hormone ( $n = 3$ ) in the effluent media.

Exposure to 3 mmol/L of 8-bromo-cAMP caused a fivefold increase in adrenocorticotrophic hormone and a concomitant 2.5- to threefold increase in luteinizing hormone and follicle-stimulating hormone concentrations in the effluent after the secretagogue treatment. Exposure to  $\geq 100$  nmol/L concentration of arginine vasopressin brought about a twofold increment in luteinizing hormone and follicle-stimulating hormone concentration and a 1.5- to 2.5-fold increase in adrenocorticotrophic hormone concentration. Exposure to 60 mmol/L of potassium chloride at the end of the superfusion experiments resulted in a significant increase in the concentrations of all three hormones in the effluent.

Three-hour incubations of the dispersed fetal pituitary cells on the extracellular matrix with 10 nmol/L of corticotropin-releasing factor, 100 nmol/L of arginine vasopressin, or a combination of both secretagogues resulted in a significant increase in adrenocorticotrophic hormone concentration in the medium as com-



**Fig. 2.** Interaction of corticotropin-releasing factor and arginine vasopressin in their effects on adrenocorticotrophic hormone, luteinizing hormone, and follicle-stimulating hormone secretion by human pituitary cells of a fetus of 17.4 weeks, in monolayer culture on extracellular matrix. Each bar represents mean  $\pm$  SD of quadruplicate wells. \*\* =  $p < 0.01$  from control.

pared to control wells incubated with medium alone ( $p < 0.01$ ), with corticotropin-releasing factor being more potent on an equimolar basis (Fig. 2). Concurrent addition of corticotropin-releasing factor and arginine vasopressin had a synergistic effect on adrenocorticotrophic hormone secretion. Luteinizing hormone and follicle-stimulating hormone secretion into the medium, however, was not significantly increased during the static incubation with corticotropin-releasing factor ( $n = 4$ ). Luteinizing hormone concentration in the medium was increased by 3-hour exposure to 100 nmol/L of arginine vasopressin or 100 nmol/L of arginine vasopressin plus 10 nmol/L of corticotropin-releasing factor as compared to the concentration in control wells and corticotropin-releasing factor-treated wells ( $p < 0.01$ ). No significant difference between luteinizing hormone or follicle-stimulating hormone responses to arginine vasopressin alone or arginine vasopressin plus corticotropin-releasing factor was detected ( $n = 4$ ). Incubations of fetal pituitary cells with 3 or 5 mmol/L of 8-bromo-cAMP brought about a significant increase in adrenocorticotrophic hormone secretion into the medium ( $n = 4$ ) but lower concentrations ( $5 \times 10^{-6}$  mol/L to  $5 \times 10^{-4}$  mol/L) failed to cause a significant increase in adrenocorticotrophic hormone secretion into the medium (Fig. 3). Luteinizing hormone secretion into the medium was significantly increased, however, by static incubation with as little as  $5 \times 10^{-5}$  mol/L ( $p < 0.05$ ) (Fig. 3). To determine

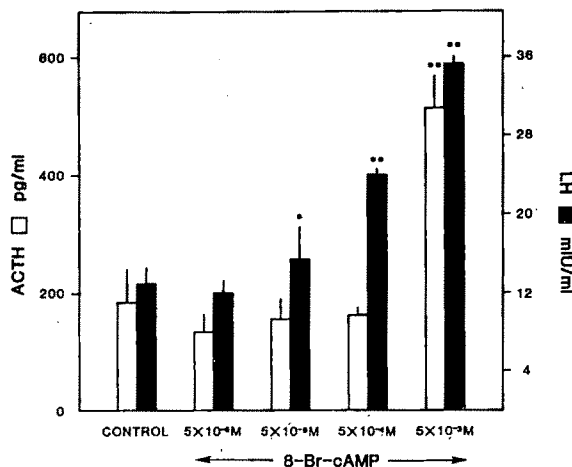


Fig. 3. The effect of different concentrations of 8-bromo-cAMP ( $5 \times 10^{-6}$  to  $5 \times 10^{-3}$  mol/L) on adrenocorticotrophic hormone and luteinizing hormone secretion by human pituitary cells of a fetus of 19 weeks, in monolayer culture on extracellular matrix. Each bar represents mean  $\pm$  SD of quadruplicate wells. \*\* =  $p < 0.01$ , \* =  $p < 0.05$  from control.

whether the influence of corticotropin-releasing factor on gonadotropin secretion was the result of contaminating peptides, the purity of both arginine vasopressin and corticotropin-releasing factor was examined by high performance liquid chromatography as described in Material and methods. Both arginine vasopressin and corticotropin-releasing factor were found to be >98% pure.

### Comment

Synthetic human corticotropin-releasing factor was found to be a potent peptide with high intrinsic activity for the stimulation of the secretion of adrenocorticotrophic hormone by human fetal pituitaries. In addition, corticotropin-releasing factor action was synergistic with arginine vasopressin as has also been found by others for rat pituitary cells<sup>18</sup> and adult women in vivo.<sup>19</sup> However, the specificity of corticotropin-releasing factor apparently is not limited to adrenocorticotrophic hormone stimulation, since gonadotropin secretion occurred after superfusion with 10 nmol/L of corticotropin-releasing factor. Among the possible explanations for gonadotropin secretion in response to corticotropin-releasing factor are (1) contamination of the corticotropin-releasing factor and/or arginine vasopressin by gonadotropin-releasing hormone, gonadotropin-releasing hormone-like peptides, or other secretagogues that cause gonadotropin secretion; (2) cross-reactivity between adrenocorticotrophic hormone and the gonadotropin radioimmunoassays; (3) a direct effect of corticotropin-releasing factor on gonadotropes as well as on corticotropes; or (4) a paracrine effect of corticotropes acting on gonadotropes.

The purity of our synthetic human corticotropin-releasing factor and arginine vasopressin was confirmed to be >98% by high-performance liquid chromatography analysis. No gonadotropin secretagogues are known to exert a biological effect at levels several times those which could have been detected by this technique. It seems unlikely, therefore, that contaminating peptides caused the observed results. No cross-reactivity was found between the adrenocorticotrophic hormone and luteinizing hormone or follicle-stimulating hormone radioimmunoassays. If corticotropin-releasing factor would have affected the gonadotropes directly to secrete luteinizing hormone and follicle-stimulating hormone, we would have expected a significant increase in gonadotropin concentrations in media of the dispersed cells after static incubation with corticotropin-releasing factor as well as in superfusion. Since we did not find such an increase, the possibility of a direct effect of corticotropin-releasing factor on the gonadotropes seems less likely. The specificity of corticotropin-releasing factor for corticotropes was demonstrated by Leroux and Pelleter<sup>20</sup> who found corticotropin-releasing factor binding to corticotropes but not to other pituitary cells using autoradiography. A paracrine effect of the corticotropes on the gonadotropes after corticotropin-releasing factor stimulation seems the most likely explanation. One possible mediator of such a paracrine effect is cAMP. Corticotropin-releasing factor was found to increase adenylate cyclase activity and cAMP production in corticotropes.<sup>20</sup> Our observation that luteinizing hormone and follicle-stimulating hormone were more sensitive to 8-bromo-cAMP exposure than was adrenocorticotrophic hormone by two orders of magnitude ( $10^{-5}$  versus  $10^{-3}$  mol/L concentrations, Fig. 3) in static incubations of dispersed cells supports the possible role of cAMP as the mediator for this paracrine effect. The intrapituitary concentrations of cAMP, presumably generated by the corticotropes after corticotropin-releasing factor stimulation, may be sufficient for stimulation of adjacent gonadotropes. However, in static incubation, the cell-to-cell interactions are two dimensional at most, and the synthesized cAMP may be diluted to concentrations too low to stimulate the gonadotropes. Another possible mediator of such a paracrine effect is  $\alpha$ -melanocyte-stimulating hormone, which has been demonstrated to induce luteinizing hormone and follicle-stimulating hormone in adult men,<sup>21</sup> normal women during the luteal phase and midcycle surge, and patients with specific types of hypothalamic-pituitary dysfunction marked by attenuated gonadotropin-releasing hormone/luteinizing hormone release or polycystic ovary syndrome.<sup>21</sup>  $\alpha$ -Melanocyte-stimulating hormone also has been demonstrated to act directly at the pituitary level in superfusion of male rat pituitaries caus-

ing the release of luteinizing hormone<sup>22</sup> but failed to induce increased luteinizing hormone release by cultured pituitary cells from immature female rats.<sup>23</sup>  $\alpha$ -Melanocyte-stimulating hormone is secreted in the rat in vivo after arginine vasopressin<sup>24</sup> and may be secreted by pituitary fragments after corticotropin-releasing factor stimulation, since it shares a common precursor with adrenocorticotrophic hormone and  $\beta$ -lipotropin.<sup>25</sup> However, Gibbs et al.<sup>25</sup> were unable to demonstrate  $\alpha$ -melanocyte-stimulating hormone secretion by human fetal pituitaries, 19 to 23 weeks of age, in response to corticotropin-releasing factor. To date there have been no reports that administration of corticotropin-releasing factor to adult humans elicits an increase in gonadotropin release. This may reflect ontogenetic changes in cell to cell interactions within the pituitary gland, some of which may disappear during the maturational process.

Finally, Matteri and Moberg<sup>26</sup> have shown recently that superfusion of ovine pituitary slices with synthetic adrenocorticotrophic hormone causes the release of gonadotropin. Corticotropin-releasing factor treatment of pituitary slices in superfusion may result in sufficiently high local levels of adrenocorticotrophic hormone to trigger subsequent release of gonadotropin, while similar amounts of released adrenocorticotrophic hormone in the culture experiments would be rapidly diluted by media to levels that are ineffective in releasing gonadotropins.

In contrast to corticotropin-releasing factor, arginine vasopressin was found to stimulate luteinizing hormone and follicle-stimulating hormone secretion in static incubations of dispersed cells as well as in superfusion. At least at the concentration tested ( $10^{-7}$  mol/L), arginine vasopressin seems to have a nonspecific secretory effect on both corticotropes and gonadotropes in the human fetal pituitary. This concentration is 7- and 2000-fold higher than the respective hypophyseal portal and peripheral blood concentrations in monkeys.<sup>27</sup> Therefore the question of whether arginine vasopressin can affect gonadotropin secretion at physiologic concentrations remains unanswered.

From this study we conclude that (1) corticotropin-releasing factor is associated with increases in adrenocorticotrophic hormone, luteinizing hormone, and follicle-stimulating hormone by human fetal pituitaries (14 to 25 weeks) in superfusion but only adrenocorticotrophic hormone secretion in static incubations of dispersed cells; (2) arginine vasopressin causes adrenocorticotrophic hormone and gonadotropin secretion by human fetal pituitaries both in superfusion and in static incubations, although at higher concentrations than corticotropin-releasing factor; (3) luteinizing hormone and follicle-stimulating hormone secretion in response to corticotropin-releasing factor is probably due to a

paracrine effect; and (4) the combination of static incubation of dispersed human fetal pituitary cells and superfusion is useful in elucidating the effects of various secretagogues on pituitary cells and in studying the ontogenesis of hypothalamopituitary interactions.

We wish to express our appreciation to Dr. Satoshi Hane for the generous supply of adrenocorticotrophic hormone antiserum and trace. We also thank Mr. Steven Zippin for technical assistance with the gonadotropin radioimmunoassays, Ms. Cynthia Voytek for her technical assistance in preparing the figures, and Ms. Mary Deyman for technical assistance with the high-performance liquid chromatography.

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# Analysis of sequential treatment protocols for endometriosis-associated infertility

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The evaluation of comparative pregnancy data in clinical studies is subject to a variety of biases. One such bias, when treated patients are retrospectively compared with untreated control subjects, results from the fact that treated patients must remain infertile from time of diagnosis to time of treatment while no such requirements is maintained for untreated control subjects. This bias is also apparent when patients undergo sequential treatments and transfer from one comparison group to another. We have eliminated this problem by applying the Mantel-Byar approach for constructing and comparing modified life tables that reflect sequential changes of treatment status. To illustrate this we applied commonly used methods of analysis as well as the Mantel-Byar method to infertile endometriosis patients attempting pregnancy. One hundred thirty consecutive patients were evaluated retrospectively, with 42 undergoing expectant management only, seven experiencing a conservative surgical procedure only, and 81 having a conservative surgical procedure after a variable period of expectant management. Use of Mantel-Byar analysis revealed no significant overall increase in pregnancy rate with conservative surgical procedures versus expectant management ( $\chi^2 = 0.225$ ,  $df = 1$ ). To further assess the potential value of conservative surgical procedures in fertility enhancement, the data were stratified by degree of endometriosis. The adjusted  $\chi^2$  was 1.621. No significant difference was noted between the two therapeutic approaches with mild disease ( $n = 35$ ,  $\chi^2 = 0.0175$ ) or moderate endometriosis ( $n = 59$ ,  $\chi^2 = 0.424$ ). Conservative surgical procedures appear to be therapeutic, however, in women with severe disease ( $n = 36$ ,  $\chi^2 = 5.12$ ,  $p < 0.05$ ). These results differed substantially from those obtained with the more biased analytic methods routinely used in clinical fertility trials. This study illustrates the utility of the Mantel-Byar approach as a valuable tool in the evaluation of response time data involving transient therapeutic states and one particularly adaptable to retrospective infertility studies involving sequential treatment approaches. (AM J OBSTET GYNECOL 1986;154:613-9.)

**Key words:** Endometriosis, infertility, sequential analysis

The cornerstone of clinical research in the field of fertility enhancement is the clinical therapeutic trial. A number of published trial designs exist, each with its own particular advantages and disadvantages.<sup>1</sup> Much attention is paid the prospective, controlled, randomized trial as being optimal under most circumstances. Unfortunately, the time requirement and cost often make this approach prohibitive to many contemporary investigators. Consequently, comparative data drawn from nonrandomized populations have frequently been analyzed in fertility research. Many pitfalls, however, await the researcher undertaking such comparisons.

A critical aspect of the therapeutic trial is the control group. The use of controls in fertility studies is partic-

ularly important in that infertility patients often have a preselection bias differentiating them from the general population.<sup>2</sup> Furthermore, infertility is rarely absolute but rather represents a relative phenomenon with a definable background pregnancy rate in the untreated patient.<sup>3</sup>

When a control group can be assembled, the evaluation of resulting pregnancy rates may still be subjected to a variety of biases. One bias commonly encountered occurs when pregnancy rates from treated patients are compared with those of an untreated control group. While patients undergoing the experimental treatment must (by definition) remain infertile from the time of diagnosis to the time the treatment is instituted, no such requirement is applied to the untreated control population. Thus poor pregnancy rates for a particular treatment entity may reflect an unfavorable selection bias that results from the "weeding out" of the most fertile women during the interval from diagnosis to treatment.

Many investigators, however, find it extremely difficult to enroll a sufficient number of comparable untreated women to make up a control group. An obvious

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**Table I.** Tally sheet for expectant management versus conservative surgical procedures (all patients)

Months	Expectant management					Conservative surgical procedures				
	<i>N</i> <sub>1</sub>	Preg-nancies	Cumulative pregnancies	Losses	Acces-sions	<i>N</i> <sub>2</sub>	Preg-nancies	Cumulative pregnancies	Losses	Acces-sions
1	123	4	4	4	0	7	0	0	0	0
2	119	7	11	29	0	7	1	1	1	22
4	90	5	16	16	0	28	2	3	2	11
5	74	2	18	10	0	37	1	4	1	8
6	64	3	21	15	0	44	1	5	2	12
7	49	2	23	5	0	54	2	7	2	3
8	44	0	23	10	0	55	1	8	1	10
10	34	0	23	5	0	64	6	14	7	5
12	29	1	24	4	0	62	6	20	6	3
13	25	1	25	1	0	59	1	21	2	0
14	24	0	25	4	0	57	1	22	1	3
16	20	1	26	2	0	59	3	25	6	0
17	18	2	28	2	0	53	0	25	0	0
18	16	1	29	6	0	53	1	26	2	4
19	10	1	30	3	0	55	1	27	12	0
22	7	0	30	5	0	43	4	31	9	0
24	2	0	30	2	0	34	1	32	5	0
26	0	0	30	0	0	29	2	34	14	0
34	0	0	30	0	0	15	1	35	1	0
36	0	0	30	0	0	14	1	36	4	0
40	0	0	30	0	0	10	1	37	10	0

solution to this dilemma arises from the realization that with many therapeutic approaches there is a distinct postdiagnosis interval when the patient is indeed untreated. Methodology allowing the use of these control intervals without the aforementioned bias of selection would significantly increase the flexibility allowable in study design and implementation. Furthermore, such methodology could easily be extended to include proper analysis of pregnancy rates in patients undergoing a series of sequential treatment protocols, ordered or nonordered.

The Mantel-Byar analysis method, an approach for constructing and comparing modified life tables that reflect sequential changes of treatment status, is applicable to just this type of situation.<sup>4</sup> This method eliminates the "time-to-treatment" bias and is capable of compensating for patient transfer from one comparison group to another (or even back and forth between groups) with a near assumption-free analysis. To illustrate the application of this technique we have analyzed a population of infertile endometriosis patients with the Mantel-Byar approach as well as with several more traditional, biased evaluations.

#### Material and methods

One hundred thirty consecutive patients with a diagnosis of infertility and endometriosis and no prior treatment were selected for study. All patients underwent a basic infertility evaluation including semen analysis, hysterosalpingogram, endometrial biopsy, and laparoscopy with chromopertubation and cervical dilation

in the secretory phase of the menstrual cycle. All patients were evaluated and had laparoscopy performed by the same physician. The diagnosis of endometriosis was made by visualization at the time of laparoscopy. Subclassification according to the degree of endometriosis at the time of laparoscopy was according to the staging system of Acosta et al.<sup>5</sup>

After the diagnosis of endometriosis, patients were offered conservative surgical procedures as treatment; those who refused were managed expectantly. Furthermore, many choosing conservative surgical procedures were evaluated expectantly for variable periods until operation could be implemented. One hundred twenty-three women experienced some duration of expectant management, during which time they attempted conception, and 88 women underwent conservative operation.

All charts were retrospectively reviewed. Patients were characterized by age, gravidity, parity, years of prior infertility, length of follow-up, conceptions, degree of endometriosis, and additional infertility factors such as ovulatory dysfunction, uterine anomalies, and male factor. Length of follow-up includes only those months where pregnancy was actively attempted.

**Statistical analysis.** Standard pregnancy rates (number of pregnancies per number of patients undergoing treatment) were calculated for the entire group as well as each subgroup when stratified by degree of endometriosis. Statistical significance was tested by means of the  $\chi^2$  test or Fisher's exact test when appropriate. In addition, monthly fecundity rates for expectant man-

Expectant management		Conservative surgical procedure		Variance	Cumulative variance
Expected pregnancies	Cumulative expected pregnancies	Expected pregnancies	Cumulative expected pregnancies		
3.78	3.78	0.22	0.22	0.199	0.199
7.56	11.34	0.44	0.66	0.396	0.595
5.34	16.68	1.66	2.32	1.202	1.797
2.00	18.68	1.00	3.32	0.655	2.452
2.37	21.05	1.63	4.95	0.939	3.390
1.90	22.95	2.10	7.05	0.968	4.359
0.44	23.40	0.56	7.60	0.247	4.606
2.08	25.48	3.92	11.52	1.289	5.895
2.23	27.71	4.77	16.29	1.419	7.314
0.60	28.30	1.40	17.70	0.413	7.727
0.30	28.60	0.70	18.40	0.208	7.935
1.01	29.61	2.99	21.39	0.727	8.662
0.51	30.12	1.49	22.88	0.373	9.035
0.46	30.58	1.54	24.42	0.351	9.386
0.31	30.89	1.69	26.11	0.256	9.643
0.56	31.45	3.44	29.55	0.452	10.095
0.06	31.51	0.94	30.49	0.052	10.147
0	31.51	2.00	32.49	0	10.147
0	31.51	1.00	33.49	0	10.147
0	31.51	1.00	34.49	0	10.147
0	31.51	1.00	35.49	0	10.147

agement and conservative operation were calculated according to the method of Cramer et al.,<sup>6</sup> and this method was also applied to stratified subgroups. The complete data set was also analyzed with the use of the two-parameter descriptive cumulative hazard function of Guzik and Rock<sup>7</sup> with the Marquardt method for nonlinear regression. Unfortunately, sample size precluded use of this approach with data stratified by severity of disease.

To implement the Mantel-Byar method of data analysis, a tally sheet was constructed (Table I). The first column of the table shows the number of months after diagnosis when a conception occurred in either group. Columns 2 through 6 represent running totals from patients with expectant management; column 2 is the total number of patients managed expectantly that month, column 3 represents the number of pregnancies during the month, the next column is the cumulative total of pregnancies up to that particular time point, and the fifth column is a total of those lost from further observation, by either achieving pregnancy, being lost to follow-up, no longer attempting conception, or switching groups. Column 6 represents accessions to the group during the month. Columns 7 through 11 represent analogous totals for the patients undergoing conservative operations. If a patient transfers from one group to another, she is tallied as a loss from observation from one group and also as an accession into the other group. Thus when a patient undergoes a conservative surgical procedure she is immediately removed from the expectant management

**Table II.**  $2 \times 2$  Table format for each time point for comparison of expectant management versus conservative surgical procedures

	Preg-nancies	Nonpreg-nancies	Total
Expectant management	$P_1$	$F_1$	$N_1$
Conservative surgical procedures	$P_2$	$F_2$	$N_2$
Total	$P_T$	$F_T$	$N_T$

group as a loss from observation and placed into the conservative surgical procedure group as an accession.

Consider now the data for any single month of follow-up where at least one pregnancy occurs: Among  $N_1$  expectantly managed women there are  $P_1$  pregnancies and  $N_1 - P_1 = F_1$  who fail to achieve pregnancy. Patients undergoing conservative surgical procedures have  $N_2$  women at the same month of follow-up, with  $P_2$  pregnancies and  $F_2$  nonconceptions. In total, there are  $N_1 + N_2 = N_T$  patients attempting pregnancy,  $P_1 + P_2 = P_T$  pregnancies, and  $F_1 + F_2 = F_T$  non-conceptions. These data can obviously be placed into a  $2 \times 2$  table with columns being pregnancy and no pregnancy, while rows represent expectant management and conservative surgical procedures (Table II). By the Mantel-Haenszel approach,<sup>8</sup> the conditional null expectation of  $P_1$  given  $N_1$ ,  $N_2$ ,  $P_T$ , and  $F_T$  is  $N_1 P_T / N_T$  with conditional variance:



**Table III.** Pregnancy rates, monthly fecundity rates, and hazard rates in conservative surgical procedures versus expectant management

	Simple pregnancy rate			Monthly fecundity rate†		Cumulative hazard rates	
	No. of patients*	Pregnancies		Months	%	Cure rate (%)	λ (%)
		No.	%				
All patients							
Conservative surgical procedure	88	37	42.0	1442	2.6	60.0	6.3
Expectant management	123	30	24.4	957	3.1	72.7	4.7
Mild endometriosis							
Conservative surgical procedures	11	5	45.5	127	3.9	—	—
Expectant management	34	18	52.9	316	5.7	—	—
Moderate endometriosis							
Conservative surgical procedures	43	22	51.2	613	3.6	—	—
Expectant management	57	12	21.1	410	2.9	—	—
Severe endometriosis							
Conservative surgical procedures	34	10	29.4	702	1.4	—	—
Expectant management	32	0	0.0	231	0.0	—	—

\*Patients undergoing conservative surgical procedures despite an initial period of expectant management are classified as having conservative surgical procedures only for calculations of simple pregnancy rates.

†Monthly fecundity rate = pregnancies per months of follow-up while attempting conception.

$$N_1P_1N_2F_T/N_T^2(N_T - 1)$$

To calculate the  $\chi^2$  statistic up to a given month of follow-up, the summation of the various parameters is required up to that point (see columns 12 through 17 of Table I). Thus  $\Sigma P_1$  = the cumulative number of pregnancies to that point in time, while the expected number (given the null hypothesis of no difference in cumulative pregnancy rates between the two treatments) is  $\Sigma N_1P_1/N_T$  and the cumulative variance is:

$$\Sigma N_1P_1N_2F_T/N_T^2(N_T - 1)$$

The calculated  $\chi^2$  statistic is:

$$\chi^2 = \frac{(\Sigma P_1 - \Sigma N_1P_1/N_T)^2}{\Sigma N_1P_1N_2F_T/N_T^2(N_T - 1)}$$

## Results

**Simple pregnancy rates.** In calculating simple pregnancy rates on the patients studied, the results can be seen in Table III. This simplistic approach yielded a significantly higher overall pregnancy rate in patients undergoing conservative surgical procedures ( $p = 0.01$ ). By stratifying the patients into mild, moderate, and severe disease, no difference was apparent in the mild disease group. However, conservative operation was noted to be significantly more effective in moderate endometriosis ( $p = 0.003$ ) and severe disease ( $p = 0.0012$ ).

**Monthly fecundity rates.** The monthly fecundity rates in the total group of patients showed no significant

difference between expectant management and conservative operation (Table III). When the data were stratified, there was still no difference noted between the two therapeutic approaches.

**Two-parameter cumulative hazard rate.** By means of the Marquardt method for nonlinear regression as applied to a two-parameter cumulative hazard function, the parameters  $c$  (cure rate) and  $\lambda$  (the monthly fecundity rate among those cured) can be calculated. With this approach, the study population demonstrated no statistically significant difference in either individual parameter or in the combined function (Table III). No stratification was possible with this analytic method because the patient numbers in the subgroups of mild, moderate, and severe disease were too small to permit estimation of the model parameters.

**Mantel-Byar analysis.** As shown in Table IV, analysis of the entire patient population resulted in a  $\chi^2$  statistic of 0.225, far below that needed to achieve statistical significance. It must be noted, however, that such a value can be skewed by uneven weight attributed to the various stages of endometriosis. To calculate an "adjusted"  $\chi^2$  value, the calculations previously illustrated were performed within each stratum as defined by endometriosis stage. These results were then summed across strata to produce an adjusted value for the entire group (which is unaffected by the variation in numbers within each stage of disease). In this study, the  $\chi^2$  was 0.0175 (not significant) for mild disease and was 0.424 (not significant) for moderate disease. For severe en-

**Table IV.** Mantel-Byar analysis of conservative surgical procedures versus expectant management

	<i>N</i>	$\Sigma P_1$	$\Sigma Exp P_1^*$	$\Sigma P_2$	$\Sigma Exp P_2^\dagger$	$\Sigma Var.$	$\chi^2$
All patients	130	30	31.51	37	35.49	10.15	0.225
Mild endometriosis	35	18	17.78	5	5.22	2.76	0.0175
Moderate endometriosis	59	12	13.40	22	20.60	4.62	0.424
Severe endometriosis	36	0	2.56	10	7.44	1.28	5.12
"Adjusted" totals	130	30	33.74	37	33.26	8.66	1.621

\*Expected cumulative pregnancies =  $\Sigma N_1 P_1 / N_T$ .†Expected cumulative pregnancies =  $\Sigma N_2 P_2 / N_T$ .

dometriosis, however, conservative operation proved significantly more beneficial in fertility enhancement ( $\chi^2 = 5.12$ ,  $p = 0.023$ ). The "adjusted" chi square for the total patient population is 1.621, still falling short of statistical significance.

### Comment

Proper analysis of the data resulting from clinical fertility trials is essential when attempting to obtain maximum information from such research. This is a nonissue to some degree when a prospective, randomized, well-controlled study with complete follow-up is carried out. In these situations a direct comparison of conception rates yields the information sought. However, this approach is not used for the vast majority of clinical trials, nor is it useful in many cases when results are compared from several investigations of slightly different design.

Simple pregnancy rate calculations have been a standard among infertility specialists for many years. While this statistic will provide a minimum success rate, it makes no provisions for length of follow-up among patients. A reported pregnancy rate of 50% has a vastly different meaning with 1 month of follow-up versus 1 year of follow-up. Furthermore, if the follow-up among study patients is variable, no adjustment is made for this. An example of these problems can be seen in endometriosis research, where the length of follow-up is generally quite prolonged for patients who undergo operation, intermediate for those treated medically, and quite brief for expectant management. Although all may produce similar success rates, the actual pregnancy rate per unit time after therapeutic intervention may differ considerably.<sup>2,9</sup> When data with "censored" patients and variable durations of follow-up are analyzed, the simple pregnancy rate should never be used for statistical purposes.

A second difficulty with simple pregnancy rates is seen when data are collected after sequential treatment protocols. If rates are reported, as we have done here, the women who switch from expectant management to conservative operation are in essence counted twice while those treated by a single modality are included only once, inflating the sample size and weighting the

information from subjects unequally. However, to classify women into their final treatment group removes expectant management "failures" and biases the evaluation against conservative surgical procedures. Thus regardless of the approach the use of simple pregnancy rates with sequential treatments is inappropriate.

The monthly fecundity rate described by Cramer et al.<sup>6</sup> corrects for this type of variable follow-up. However, this use of a one-parameter exponential model has significant limitations in its assumptions of a constant rate of conception during a period of time and a 100% success rate given an infinite length of follow-up. While this model is adequate for parous artificial insemination by donor patients,<sup>10</sup> its utility for many infertility populations (including those with endometriosis) has been shown inadequate.<sup>7,10</sup>

In an attempt to correct the deficiencies of the Cramer model, Guzick and Rock<sup>7</sup> have developed a two-parameter exponential model with the Marquardt nonlinear regression method. A major drawback with this regression method, however, is its assignment of equal weight to all time points along the life table, a situation that in most cases does not correspond to the distribution of the data. This was later corrected by a model of their own design.<sup>11</sup> Both approaches have been shown to correlate well with pregnancy data after treatment of endometriosis. Unfortunately, the greatest limitation of this method seems to be the requirement for sample sizes in excess of 30 to 40 per group, a difficult stipulation to reach in many infertility studies. Furthermore, no correction is made for the bias of selection by delay in instituting treatment or for patients undergoing multiple treatment protocols. Finally, the modified nonlinear regression method of Guzick, while correctly weighting all data points, is extremely sensitive to deviations from the exponential model it is based on (and for this reason could not be applied to the data in this study). Thus, while this approach is valid for the select investigation, its applicability in many clinical situations is limited.

The Mantel-Byar approach eliminates many of the aforementioned problems. It was originally designed to compensate for the bias of the diagnosis-to-treatment interval and is particularly useful in analyzing patients

who transfer from one treatment group to another or even back and forth between groups. The analysis is nearly assumption free and any stratification that improves the previously mentioned parametric type of analysis can also be applied to this method.

While it has been stated that the Mantel-Byar approach is nearly assumption free, one implicit assumption (as with all the previously mentioned methods) is that past history before being placed into a treatment group is of no consequence. This assumption may fail to hold; a patient may be more likely to respond to present treatment because of previous treatments. While there are ways for correcting for this situation,<sup>1</sup> the question of validity of the above assumption should be asked before the use of this technique.

It is worth noting that Yates' correction for continuity was used in the  $\chi^2$  calculation by Mantel and Byar; yet we have chosen to omit this from our calculations. While some have advocated the use of this correction,<sup>12,13</sup> many others have advised against it as unnecessary, too conservative, and decreasing the accuracy of probability statements.<sup>14-16</sup> In this analysis the inclusion of the correction for continuity would not have altered any results, consistent with the advice of Brown<sup>17</sup> that "if the P value is so close to the rejection region that the use or non-use of Yates' correction would change the conclusion, the research should probably be considered suggestive but not conclusive."

Care must be taken, in reviewing the results of this study, not to draw inappropriate conclusions. The fact that no advantage was found in the use of conservative surgical procedures in patients with moderate endometriosis does not mean that operation is inappropriate for the infertile patient with moderate disease. Rather, it implies that there is no justification for grouping all patients with moderate endometriosis (by the Acosta classification) as in need of operation. It is quite plausible that there is a subset of the moderate stage (or even mild stage) that would benefit from conservative operation. Unfortunately, the Acosta system does not effectively sort out this subset if it does exist. Whether a more recent classification model<sup>18,21</sup> would more effectively delineate which patients would benefit from operation and which would not is open to question.

Finally, even the conclusion that fertility rates with severe endometriosis are improved with conservative operation is open to debate. While the results appear to demonstrate a significant difference between the two approaches, it must be remembered that multiple comparisons were made in the analysis. Thus the overall p value desired to achieve significance should be adjusted downward, either by the Bonferroni correction or actual tables of revised alpha levels for multiple tests.<sup>17</sup> Correction in this manner produces a required p value of 0.017, slightly below the value of 0.023 obtained. In

addition, since the study was not a randomized clinical trial, unrecognized baseline differences between the groups with severe endometriosis could have accounted for some increase in the difference in pregnancy outcome. For these reasons, the significance achieved with severe disease can only be looked upon as suggestive rather than conclusive.

In conclusion, we have presented a method for analyzing data from sequential treatment protocols with the use of an investigation of endometriosis therapy to enhance fertility. The Mantel-Byar method is simple, powerful, and adaptable. Results from the analysis are shown to differ considerably from conclusions drawn by more traditional statistical approaches, with conservative operation proving to be of benefit only in the patient with severe endometriosis. We believe the Mantel-Byar method to be a worthwhile approach to the analysis of fertility trials.

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## Fetal response to vibratory acoustic stimulation in periods of low heart rate reactivity and low activity

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The fetal response to vibratory acoustic stimulation during periods of low fetal activity and low fetal heart rate reactivity was studied in 10 healthy pregnant women at term. In each case, two periods of low reactivity were studied. Consecutive cases alternated: The vibratory acoustic stimulus was applied 10 minutes after the first nonreactive period in half of the cases; the remainder were stimulated during the second nonreactive period. The unstimulated period served as a control. After vibratory acoustic stimulation the baseline fetal heart rate, the mean number of fetal heart rate accelerations, and, the number of fetal movements were significantly increased compared with values in the control nonstimulated periods ( $p < 0.0001$ ). This consistent response to vibratory acoustic stimulation may prove to be clinically useful in altering periods of low reactivity observed during nonstress testing of normal fetuses. (*Am J Obstet Gynecol* 1986;154:619-21.)

**Key words:** Vibratory acoustic stimulation, fetal reactivity

Nonstressed fetal heart rate (FHR) monitoring is widely used in clinical practice as a test of fetal well-being.<sup>1,2</sup> A nonreactive FHR pattern may be indicative of poor fetal condition, and in such cases shaking of the fetus through the maternal abdomen has been tried in an attempt to alter the FHR to a reactive pattern, thereby demonstrating its state of health.<sup>2</sup> Nevertheless, recent controlled studies<sup>3,4</sup> have indicated that such external physical stimulation does not "wake" the fetus. This lack of responsiveness may signify the inability of external stimuli to alter the fetal pattern of low activity and low FHR variability. Alternately, it may indicate that this particular "shake" stimulus is not an adequate one to use for this purpose.<sup>3</sup>

The aim of this study was to examine, during periods of low activity and low FHR reactivity ("quiet periods"), the effect of a different type of stimulus, a vibratory acoustic noise source.<sup>5</sup> Changes in fetal parameters were observed by use of continuous heart rate monitoring and real-time ultrasonography.

### Patients and methods

Ten healthy women with normal pregnancies at term were studied. Their subsequent labor and the neonatal outcome were normal. The studies were carried out in a quiet room with the women in bed in a semilateral position. The FHR was continuously recorded by the use of an external ultrasound transducer (Hewlett-Packard, Model 8040 A). Fetal movements were observed with real-time linear array scanning (Aloka). The stimulus used was a vibratory acoustic noise source (Electrolarynx, Model 5B, Western Electronic; audible sound 750 to 1000 Hz, vibrations between 110 and 200 Hz, output intensity 110 dB). This stimulus was firmly applied for 5 seconds on the maternal abdomen overlying the fetal head.

In each case two 25-minute periods were studied, each period beginning with a state of low heart rate variability and low activity of at least 10 minutes duration. Consecutive cases alternated: In five women vibratory acoustic stimulus was applied 10 minutes after the beginning of the first "quiet" phase. The remaining five women were stimulated during the second "quiet" phase. The 10 minutes preceding and 15 minutes after the stimulation were compared with the respective 25 minutes of the "no stimulus," control "quiet" periods. A state of low FHR reactivity and low activity was de-

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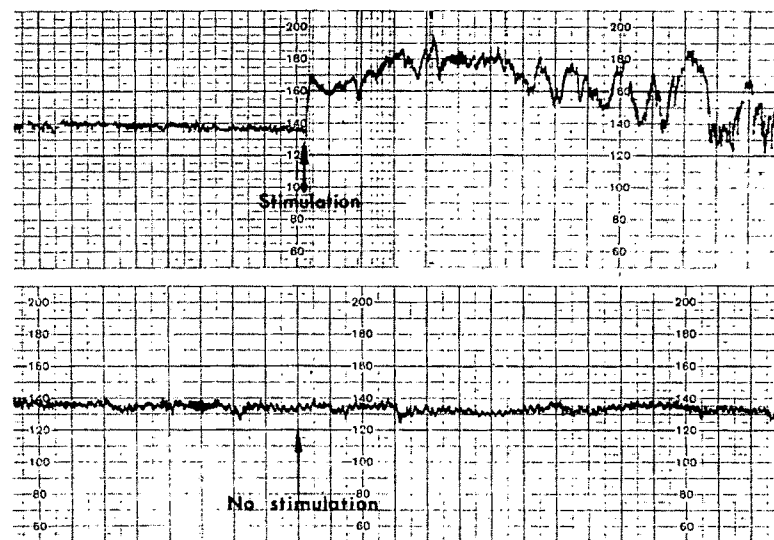


Fig. 1. Fetal heart rate recordings demonstrating the response to vibroacoustic stimulation (*top*) and a control period with no stimulation (*bottom*). (Paper speed = 1 cm/min.)

finer by the absence of fetal movements and FHR accelerations as well as decreased baseline long-term variability (amplitude range < 6 bpm). For detailed statistical analysis, the total study time, as described above, was divided into 5-minute intervals (see Fig. 2). For each time interval the following parameters were calculated and compared: (1) basal FHR (average of 1-minute intervals); (2) number of FHR accelerations (a rise from baseline of 15 bpm or more, lasting at least 15 seconds but not longer than 60 seconds); (3) number of ultrasonically detected gross fetal movements. Student's *t* test was used for statistical analysis.

### Results

After the vibratory acoustic stimulation there was an abrupt increase in baseline FHR and number of FHR accelerations as well as an increase in the number of fetal movements. In contradistinction to this, in the control, nonstimulated periods such immediate change did not occur (Fig. 1) and a spontaneous alteration to a reactive FHR pattern was observed after a mean duration of 27 minutes of nonreactivity. The mean baseline FHR, number of FHR accelerations, and number of fetal movements in the stimulation and control periods are shown in Fig. 2. The three studied parameters did not differ significantly when the initial 10 minutes of observation of the control and study periods were compared. After vibratory acoustic stimulation, the mean numbers of FHR accelerations and fetal movements were significantly increased throughout the 15 minutes of observation, compared with values in the respective control periods ( $p < 0.0001$ ). The baseline FHR, which was significantly increased in the initial 5 minutes after stimulation ( $p < 0.0001$ ), returned dur-

ing the last 10 minutes to levels that were not significantly different when compared with control values (Fig. 2).

Within the stimulated study periods the mean numbers of FHR accelerations and fetal movements were significantly increased in all poststimulation intervals as compared with values in the prestimulation period ( $p < 0.0001$ ). The baseline FHR was significantly elevated in the first and second poststimulation intervals ( $p < 0.0001$  and  $p < 0.05$ , respectively) but returned to prestimulation levels in the third poststimulation 5-minute interval.

### Comment

The presence of behavioral states during fetal life has been suggested by various authors.<sup>6,9</sup> Observation of the FHR pattern in conjunction with fetal activity allows a distinction of periods of low activity and low heart rate variability, which resembles quiet sleep in the newborn infant.<sup>7</sup> Proper interpretation of the FHR nonstress test requires that a distinction be made between low variability and low activity of a "sleeping" healthy fetus and that signifying ill health. Recent controlled studies have indicated that external physical stimulation, such as shaking of the maternal abdomen, which is often applied in clinical practice, does not contribute to this differentiation.<sup>3,4</sup>

The present study demonstrates that when a vibratory acoustic noise source is applied to healthy fetuses during periods of low FHR reactivity and low activity, a statistically significant increase in basal FHR, number of FHR accelerations, and observed fetal movements is induced. The sustained change of fetal movements and number of accelerations, which remained significantly

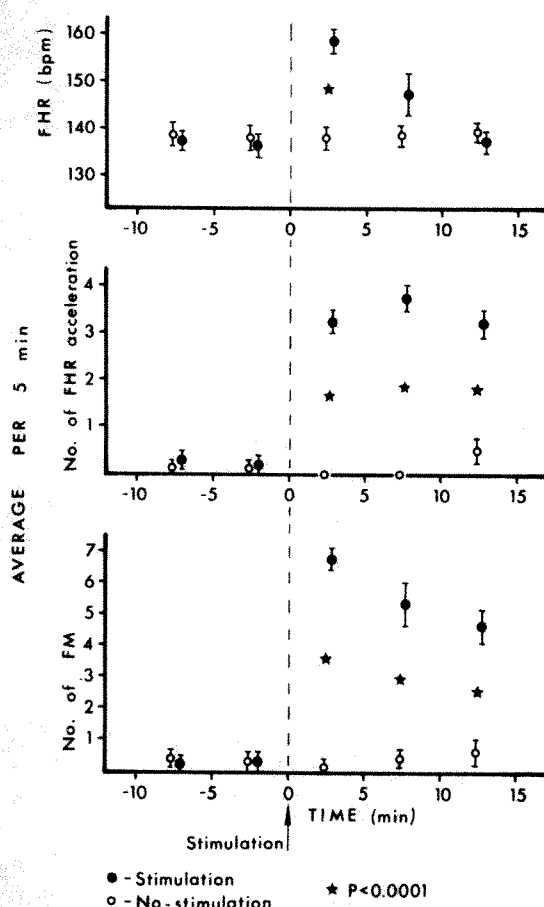


Fig. 2. The mean  $\pm$  SE of baseline FHR (*top*), number of FHR accelerations (*middle*), and number of fetal movements (*bottom*), in the stimulation and nonstimulation periods.

greater in each of the 5-minute intervals after stimulation, demonstrates that we are not observing only an isolated and short-lived response to the stimulation.

The consistency of FHR response to external sound has been challenged, with the inconsistent response of the 1- to 3-day-old newborn infant to sound given as a supporting example.<sup>10</sup> The vibratory acoustic noise source used in the present study does induce a consistent alteration from a state of low fetal activity and low reactivity to that of high fetal activity and heart rate

reactivity. Our findings suggest that the consistency of the fetal response may depend on the specific qualifications (intensity, frequency) of the stimulus used. This observed response to vibratory acoustic stimulation supports the recent interest in its use as a test of fetal well-being (Paul RH, personal communication).<sup>11,15</sup>

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# Intravascular transfusion in utero: The percutaneous approach

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A severely Rh-isoimmunized pregnancy is described in which an intrauterine transfusion of blood was given by the intravascular route directly into an umbilical vessel. Fetoscopy was not used, and the procedure was performed percutaneously under direct ultrasound visualization. (AM J OBSTET GYNECOL 1986;154:622-3.)

**Key words:** Rh isoimmunization, intrauterine transfusion

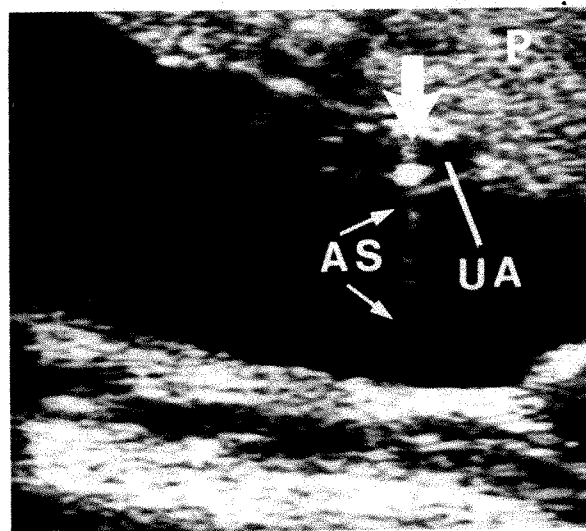
For more than 20 years, intraperitoneal transfusions have been used in the treatment of fetuses affected with severe rhesus isoimmunization. The success of this type of transfusion, however, has been limited by its relative lack of effectiveness in hydropic fetuses as well as by trauma associated with the procedure. Direct intravascular transfusions in utero have been performed under fetoscopic visualization,<sup>1</sup> but this procedure requires significant technical dexterity. Recently, pure samples of fetal blood have been safely obtained by percutaneous puncture of the umbilical cord under ultrasonic guidance.<sup>2</sup> This report describes a case in which a severely erythroblastotic fetus was transfused intravascularly by the percutaneous approach.

## Case report

A 29-year-old woman, para 1-0-0-1, blood type A, Rh negative, presented with anti-D and anti-C antibody titers of 1:256 and 1:32, respectively, at 17 weeks' gestation. Serial amniocenteses revealed a gradually rising trend in the amniotic fluid delta optical density at 450 nm until 31 weeks, when a steep rise into zone III of the Liley curve was noted. At this time, there was no sonographic evidence of fetal hydrops, and the lecithin/sphingomyelin ratio was 1.3 with no phosphatidylglycerol present.

After extensive discussion with the parents, it was decided to aspirate a sample of fetal blood to assess the degree of anemia, with therapeutic intervention by direct intravascular transfusion of blood if indicated.

After the mother was sedated, under direct ultrasonic visualization, a 22-gauge needle was introduced transplacentally, and the needle tip directed into one of the umbilical arteries near its insertion into the pla-



**Fig. 1.** Sonogram at the time of intravascular transfusion (arrow points to needle tip; UA = umbilical artery; AS = acoustic shadowing; P = placenta).

centa (Fig. 1). The blood aspirated was immediately analyzed on a Coulter counter and confirmed to be 100% fetal. The hematocrit was 14%, bilirubin 6.9 mg/100 ml, and blood group O, Rh-positive. Packed, washed, irradiated O-negative red blood cells were then transfused directly through the needle at a rate of approximately 1 ml/min. After a total of 38 ml of blood was administered the hematocrit was raised to 30%. At the conclusion of the procedure, fetal cardiac activity was normal, and there was no ultrasonic evidence of a placental hematoma or bleeding.

Fetal pulmonary maturity was documented at 33.5 weeks, and after a failed induction of labor, a 2300 gm female infant with Apgar scores of 9 and 9 at 1 and 5 minutes was delivered by cesarean section, 18 days after the intrauterine transfusion. Cord blood hematocrit and bilirubin were 23% and 7.4 mg/100 ml, respectively, and 50% of the cells were demonstrated to be adult on Kleihauer-Betke analysis. The neonate required a total of three double-volume exchange transfusions and was discharged at 19 days of age.

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### Comment

For more than a decade, fetoscopy has been the most widely used method for sampling fetal blood. Until 1981, fetoscopic blood sampling was performed entirely for diagnostic purposes, but with the first intravascular transfusion, this route of entry into the fetal circulation was exploited for therapeutic purposes.<sup>1</sup> In 1983, a series of 66 cases was presented in which uncontaminated fetal blood was obtained by the ultrasonically guided percutaneous introduction of a 20-gauge needle directly into the umbilical vein near the placental insertion of the cord.<sup>2</sup> This approach was used in the case we have presented.

Intravascular transfusion in utero is undoubtedly more technically exacting than the intraperitoneal instillation of blood. Nevertheless, this approach is inherently superior in some respects. By quantifying the preprocedure hematocrit inappropriate transfusions can be avoided, and a posttransfusion hematocrit can verify that an optimal amount of blood has been administered. Furthermore, direct introduction of blood into the fetal vascular compartment avoids the uncertainty of blood being absorbed from the peritoneal cavity, particularly in hydropic fetuses.

In specific cases of severe Rh sensitization, the arguments for intravascular transfusion can be quite compelling. In this case, amniotic fluid studies suggested severe Rh sensitization and an immature pulmonary

profile at 31 weeks. While ultrasound examination showed no evidence of hydrops, knowing that the fetal hematocrit was 14% convinced us that a transfusion was necessary, and having performed one, we were able to allow this fetus to remain in utero for an additional 2.5 weeks.

Having decided that in our case an intravascular transfusion was preferable to an intraperitoneal transfusion, we chose to use the percutaneous rather than the fetoscopic approach. Our experience with diagnostic blood sampling in the second and third trimesters by the percutaneous technique led us to believe that it would be less traumatic and easier to accomplish than transfusion under fetoscopic visualization. Performance of the transfusion in this patient proved to be quite uneventful, and we believe that this approach should be given serious consideration when an intravascular transfusion in utero is thought to offer advantages over the traditional intraperitoneal procedure.

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## Abdominal rescue after entrapment of the aftercoming head

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Entrapment of the aftercoming head during breech delivery is generally perceived as a complication that must be resolved by a vaginal extraction procedure. The successful rescue by classical cesarean section described in this report was performed for entrapment of the deflexed fetal head following delivery of the body and the arms. (*AM J OBSTET GYNECOL* 1986;154:623-4.)

**Key words:** Breech delivery, abdominal rescue, entrapment of aftercoming head

Entrapment of the aftercoming head by the pelvis or the cervix is a dreaded risk of breech delivery. Traditionally, this complication has been managed by vaginal

extraction. However, a recent case in our service was successfully resolved by abdominal rescue.

### Case report

S. V., a 16-year-old Hispanic primigravida, was admitted on June 7, 1985, at 33 weeks' gestation, with a history of painless leaking of amniotic fluid. Twin gestation with breech and transverse presentations, without discordance, was diagnosed earlier by ultrasound. A sterile vaginal examination at 11:45 AM unexpectedly

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revealed full cervical dilatation with a complete breech presenting at the +3 station.

Preparations for both cesarean section and vaginal breech delivery immediately began. By 12 o'clock, the operating team was ready. However, the breech was at the +5 station at this time. Therefore, a lower segment cesarean section was considered impractical. With knowledge that the pelvis was adequate but without information about the flexion of the head, it was decided to attempt vaginal delivery.

Following episiotomy, with five uterine contractions, the body was delivered spontaneously. With the right scapula within view, the posterior (left) and, after rotation of the body clockwise, the right arms were delivered by the classical technique at 12:16 PM. As delivery by Mauriceau's maneuver was attempted, the head was found fully deflexed and the mouth beyond reach. The second twin hindered external manipulation. Two attempts to achieve delivery by traction failed. Because it was felt that stronger traction would risk fetal injury, the attempt was abandoned at 12:17 PM.

The assistant was instructed to elevate the body of the fetus to reduce cord compression. The anesthesiologist initiated rapid sequence induction with Pentothal and succinylcholine. To aid relaxation 0.5% halothane with 100% oxygen was given. At 12:18 PM, the operation was begun. With the exception of the peritoneal incision, only a scalpel was used before delivery. The uterus was entered through a longitudinal corporeal incision that involved a portion of the overlapping placenta. The head of the fetus, with the occipitomentral diameter still transverse, was grasped. Supporting the traction, one assistant pushed the body upward into the pelvis. Some degree of shoulder dystocia (in reverse) was encountered before the fetus was extracted at 12:19 PM, head first through the abdominal incision. The second twin was delivered seconds later. Prophylactic administration of ampicillin was initiated and the operation was concluded in a routine manner.

The 1-minute Apgar score for both twin A (male, 2050 gm) and twin B (male, 1720 gm) was 3. At 5 minutes, the scores were 7 and 6, respectively. Twin A had normal initial blood gas values. However, bloody spinal taps (red blood cell count = 518,000/mm<sup>3</sup>) necessitated repeated computerized axial tomographic scans that indicated minimal transitory subarachnoid hemorrhage. Hyperbilirubinemia (peak 12.8 mg/100 ml) was noted. The neurological status remained normal. Twin B displayed mild metabolic acidosis at birth

and moderate anemia that required transfusion. His neuromuscular maturity assessment (33 weeks) was lower than that of twin A (35 weeks). The twins had identical blood groups. Both babies made good progress and were discharged on June 27, 1985.

The mother had a febrile postoperative course. Hemoglobin dropped from 12.5 to 9.5 gm. However, blood transfusion and antibiotic treatment ensured satisfactory recovery. She left the hospital on June 15, 1985.

### Comment

Only 8 days after this "abdominal rescue," a publication appeared<sup>1</sup> that contained anecdotal reference to three similar operations performed by Dr. William Stromme, an anonymous resident, and an unnamed female general practitioner in California. These differed from ours in only two respects: (1) the rescue preceded the delivery of the shoulders and arms and (2) the time intervals between failed extraction and abdominal delivery exceeded ours.

It facilitated quick intervention that our patient was slim and the operating team was prepared. In this situation, the fetus could be extracted abdominally only about 2 minutes after the failed Mauriceau procedure. The outcome suggests that cord compression was reduced by avoidance of undue force during attempts at extraction and by the maneuver we used before delivery.

Abdominal rescue carries risk of its own. We had to proceed with "crash" anesthesia, without aseptic precautions, with no immediate attention to hemostasis, through a classical incision, and at a speed that increased the risk of injury. Nonetheless, our experience suggests that when unexpected difficulty with delivery of the upper body arises during breech delivery, abdominal rescue may offer a better chance for fetal salvage than either symphysiotomy<sup>1,2</sup> or perseverance with vaginal extraction beyond the point of no return.

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# Prevalence of hepatitis B surface antigen among women receiving prenatal care at the Palm Beach County Health Department

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Pregnant women receiving prenatal care at the maternity clinics of the Palm Beach County Health Department were tested for hepatitis B surface antigen. Routine screening of pregnant women for the antigen is discussed. The results of this study indicate the need for routine screening of our medically indigent pregnant population. (AM J OBSTET GYNECOL 1986;154:625-6.)

**Key words:** Hepatitis B, pregnancy, medically indigent, risk factors

Transmission of hepatitis B virus from pregnant women who either have an acute hepatitis B virus infection or who are carriers of chronic hepatitis B surface antigen is one of the most efficient modes of transmitting the virus to their newborn infants. The availability of both passive and active immunization against type B hepatitis provides an opportunity to prevent perinatal transmission and, in turn, decreases an endemic source of this infection.<sup>1,2</sup>

This study was designed to look at the rate of occurrence of hepatitis B surface antigen in mothers attending a county health department's maternity program and to detail the presence or absence of high-risk factors to determine screening protocol for that population.

## Material and methods

Between January 1, 1984, and September 30, 1984, all maternity patients attending their first prenatal appointment at one of the Palm Beach County Health Department's Clinics had their blood tested for hepatitis B surface antigen. The first 200 patients screened had detailed chart reviews focusing on the 10 groups with risk factors for acquiring hepatitis B. These groups are as follows<sup>1</sup>: (1) Those with acute or chronic liver disease. (2) Those who were born in Asian, African, Caribbean, or South American countries; also those of Pacific Islander or Native Alaskan descent. (3) Those who were rejected as blood donors because they were

found to be hepatitis B surface antigen positive. (4) Those who work or reside in institutions for the mentally retarded. (5) Those who work or receive treatment in a hemodialysis unit. (6) Those who have been illicit injectable drug users. (7) Those who are household contacts of persons with acute or chronic hepatitis B. (8) Those with hereditary or acquired disorders requiring repeated transfusions. (9) Those who are health professionals with an occupational exposure to blood. (10) Those with multiple episodes of venereal disease.

Those patients who tested positive for hepatitis B surface antigen were interviewed by a physician or a nurse epidemiologist as to the presence or absence of such risk factors. The method used for hepatitis B surface antigen determinations was the Abbott Auszyme, an enzyme immunoassay technique. Results were read on a dual wavelength spectrophotometer. All tests were confirmed with use of control subjects. Testing was done at the Palm Beach County Health Department laboratory.

## Results

The population under study is the service population of the four coastal county health department centers. All the patients are medically indigent. Of the 741 maternity patients screened for hepatitis B surface antigen, eight were positive for hepatitis B virus, which was a rate of 1.1%. Of these eight patients only three (38%) presented with a risk factor of hepatitis B virus infection. The remaining five (62%) did not meet any high-risk criteria.

Close scrutiny of the first 200 patients screened revealed an age range from 14 to 41 with an average age of 28. Gestational age on initial prenatal visit ranged from nine to 41 weeks with an average of 25. The number of American black and American white women

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studied was essentially equal. The majority of patients from the Caribbean were from Haiti.

Three of the 200 patients had a positive hepatitis B surface antigen test, giving a rate of 1.5%. Two of these three patients had a risk factor for hepatitis B virus infection. Forty-eight (25%) of the 197 antigen-negative patients also had a risk factor for hepatitis B virus infection whereas 149 (75%) did not admit to any high-risk factors. Only one risk factor was found in any one patient. The sensitivity of using the 10 risk factors in identifying hepatitis B surface antigen-positive patients is 0.67; the specificity is 0.76. The predictive value of a positive test is 0.04 and of a negative test, 0.99.

#### Comment

The present recommendations for selective screening of maternity patients at high risk for hepatitis B virus infection is documented. The prevalence of hepatitis B surface antigen in the United States is 0.1% to 0.5%. In the population studied the prevalence is 1.1%, and more than half of the patients with hepatitis B surface antigens would have gone undetected if the recommended selective screening had been done. If selective screening had been done, over 25% of mothers would have been tested; yet 67% of cases would not have been detected. The presence of one or more risk factors in this population is a poor predictor of hepatitis B surface antigen positivity.

The question of infectivity and the possible need for hepatitis B eAg screening arises. The presence of hepatitis B eAg conveys a higher infant attack rate. Yet even in the absence of the antigen and/or the presence of anti-hepatitis B eAg, infection may lead to a fatal outcome. Also, if the mother is a carrier, risk of acquiring the infection because of chronic exposure is present. Hepatitis B immunoglobulin and vaccine are recommended for the infants of all mothers with hepatitis B surface antigens regardless of e antigen status.

Screening cannot be selective in this population. The economic consequence of hepatitis B virus infection has been documented. The cost-effectiveness of vaccination has also been documented. For populations with attack rates of 1% to 2%, vaccination is felt to be cost-effective or cost saving. Our results support the usefulness of routine screening of mothers for hepatitis B surface antigen at the Palm Beach County Health Centers.

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## Cushing's syndrome in pregnancy: A case report and literature review

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The thirty-third instance of pregnancy and Cushing's syndrome is presented and the literature is reviewed. There is a poor fetal prognosis without definitive treatment of Cushing's syndrome during pregnancy. Maternal complications are common with adrenal adenomas, 44% developing pulmonary edema and 100% developing hypertension. Prompt diagnosis and early treatment are necessary. (AM J OBSTET GYNECOL 1986;154:626-8.)

**Key words:** Pregnancy, Cushing's syndrome, pulmonary edema, premature labor

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A case of Cushing's syndrome in pregnancy is presented.

#### Case history

The patient, a 24-year-old, 230-pound, white woman, gravida 4, para 1-0-2-1, was transferred on May 4, 1984,

**Table I.** Reported cases of Cushing's syndrome in pregnancy

Series	Year	Case No.	Pregnancy No.	Abnormality	Treatment	Fetal outcome	Maternal complications
Hunt	1953	1	1	H	0	SA at 20 wk	0
			2	H	0	PB	0
			3	H	0	PB	0
			4	H	0	PB	0
		2	5	H	0	SB	1
			6	C	0	TB	2,3
			7	C	0	SA at 12 wk	0
			8	C	0	TB	0
Greenblatt	1959	4	7	C	0	TB	4
Birke	1959	5	9	C	0	SA at 10 wk	0
MacGregor	1960	6	10	H	0	TB	2,5,6
Bergman	1960	7	11	H	BA at 12 wk	TB	0
Andreoli	1962	8	12	H	BA at 20 wk	TB	0
Litowski	1962	9	13	H	BA at 12 wk	TB	0
Eisenstein	1963	10	14	A	UA at 18 wk	PB	2
Molinatti	1964	11	15	H	UA at 12 wk	PB	0
Kreines	1964	12	16	A	0	SB	2,7,8
			17	A	0	PB	2,8
			18	A	0	SB	2,7
			19	C	0	PB	2,5,9,10
Lopez	1964	14	19	C	0	TB	8
Bank	1965	15	20	C	0	SA at 20 wk	0
Parra	1966	16	21	A	0	SB	2,5
			22	A	0	PB	2,4,5,7,11
Bowman	1966	17	23	A	0	PB, AI	2
Kreines	1971	18	24	A	0	PB	8
Lee	1972	19	25	H	0	PB	2
Calodney	1973	20	26	H	0	TB	2,7,8
Grimes	1973	21	27	A	UA at 16 wk	TB	2
Anelavis	1976	22	28	A	0	PB	2,8
Keegan	1976	23	29	A	0	TB	8
Anderson	1976	24	30	H	PI at 24 wk	SA at 24 wk	2,7,8,12
Blumsohn	1978	25	31	A	UA at 21 wk	PB	2,8
Reschini	1978	26	32	A	0	TB	8
Check	1979	27	33	H	0	PB, CP	2,8
Khakoo	1982	28	34	A	0	TB	2,13
Gormley	1982	29	35	A	M	PB	2,7
Verdugo	1982	30	26	A	0	PB	0
Liu	1983	31	37	H	0	TB	2
Margulies	1983	32	38	A	0	SA at 10 wk	0
Present case	1983	33	39	A	0	SA at 10 wk	0
			40	A	0	PB	2,5,7,10,12
			41	A	0		

H = Hyperplasia; C = carcinoma; A = adenoma; BA = bilateral adrenalectomy; UA = unilateral adrenalectomy; PI = pituitary irradiation; SA = spontaneous abortion; PB = premature birth; SB = still birth; TB = term birth; AI = adrenal insufficiency; CP = cleft palate; M = metyrapone. 1 = Retained placenta with hemorrhage and shock; 2 = hypertension; 3 = no lactation; 4 = compression fractures; 5 = preeclampsia; 6 = Addisonian crisis; 7 = pulmonary edema; 8 = abnormal oral glucose tolerance test; 9 = third-degree heart block; 10 = death; 11 = kidney stones; 12 = ulcer.

from a level 1 center at 25 weeks' gestation because of a blood pressure of 180/140 mm Hg.

Her history revealed no previous hypertension or significant medical problems. She had a vaginal delivery at term, at the age of 16, when she weighed 94 pounds, and had two spontaneous abortions in the last 3 years. During the last 8 years she had gained 130 pounds and had noticed increased fatigue and weakness, hirsutism, easy bruising, and acne. Physical examination showed the classic signs of Cushing's syndrome. She was placed on a regimen of 2 gm of methyl dopa and 200 mg of hydralazine per day, while testing for Cushing's syndrome was begun. The 8:00 AM cortisol level was 23 mg/dl and showed no diurnal variation. An overnight 1 mg dexamethasone suppression screen and a 4-day dexamethasone suppression test, with 2 and 8 mg daily

doses, did not suppress the baseline urinary 17-hydroxycorticoid level of 20 mg/24 hr. During the testing, mild superimposed preeclampsia progressed to severe preeclampsia and the HELLP syndrome (syndrome of hemolysis, elevated liver enzymes, and low platelet counts), necessitating delivery. A low vertical cesarean section was done and a 27-week, small for gestational age, 660 gm breech infant was delivered with the patient under epidural anesthesia. A central venous pressure reading prior to administration of the epidural anesthetic was 18 mm Hg. A left adrenalectomy revealed a 1.5 cm adenoma. Intraoperatively and postoperatively, the patient was placed on a regimen of high levels of steroids, as recommended by Hardy,<sup>1</sup> to prevent an Addisonian crisis. On day 5, the patient developed a wound infection with some skin breakdown



around the inferior retention sutures. Cortisone was decreased from 37 to 25 mg/day. On day 11 she had hematemesis with a blood pressure of 30/0 mm Hg. Nasogastric irrigation and transfusion were unable to stabilize the patient and an antrectomy (Billroth II procedure) was performed for anterior and posterior gastric ulcers, both penetrating through the wall, with a bleeding gastroduodenal artery. She received 24 units of blood and 7 units of fresh-frozen plasma. Her post-operative course was complicated by pulmonary edema, adult respiratory distress syndrome, recurrent bleeding and fistulization from the anastomosis site, pneumonia with *Candida* and *Klebsiella* organisms, and oxygen toxicity of the lungs. Cardiopulmonary arrest and death ensued.

Follow-up of the baby revealed a grade 4 intraventricular hemorrhage and bronchopulmonary dysplasia 6 months later.

#### Comment

This is the thirty-third case report and the first report of death in a patient with Cushing's syndrome in pregnancy. A review of the literature in Table I reveals that maternal complications are common. Seven of 16 (47%) patients with adrenal adenoma developed pulmonary edema, and every patient whose pregnancy progressed

to the first trimester experienced hypertension. Maternal complications were much less common in patients with adrenal hyperplasia, with only one of 12 patients developing hypertension. There was a high incidence of prematurity with only 13% term births. This may be due to 17-hydroxylase induction in high-cortisol states, described by Liggins.<sup>2</sup>

Early diagnosis and prompt treatment are of vital importance. Prompt operation or pituitary irradiation during pregnancy yielded five term births, two premature infants, and one spontaneous abortion, giving a 62.5% term birth rate. Medical treatment was attempted by Gormley with metyrapone. This treatment appears to be an especially viable alternative in poor surgical candidates or in stabilizing patients prior to operation. Prophylactic cimetidine may also be advisable because of the high incidence of gastric ulcers in these patients.

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A complete list of references is available on request from Dr. Koerten.

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## Amniotic fluid ferning in early gestation

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Ferning of amniotic fluid was studied in early pregnancy. Arborization was demonstrated during the interval of 6½ to 16 weeks by flame-drying. Air-drying yielded a spectrum of crystallization from none prior to 8 weeks to full ferning by 16 weeks. Technical differences are stressed. (AM J OBSTET GYNECOL 1986;154:628-30.)

**Key words:** Amniotic fluid, ferning, crystallization, early gestation

Premature rupture of membranes during the second trimester of pregnancy confronts the obstetrician and the patient with a dilemma. The high incidence of fetal pulmonary hypoplasia, deformations from amniotic bands, and maternal infection requires serious in-depth discussion with the patient about whether to interrupt the gestation or risk the consequences of continuing

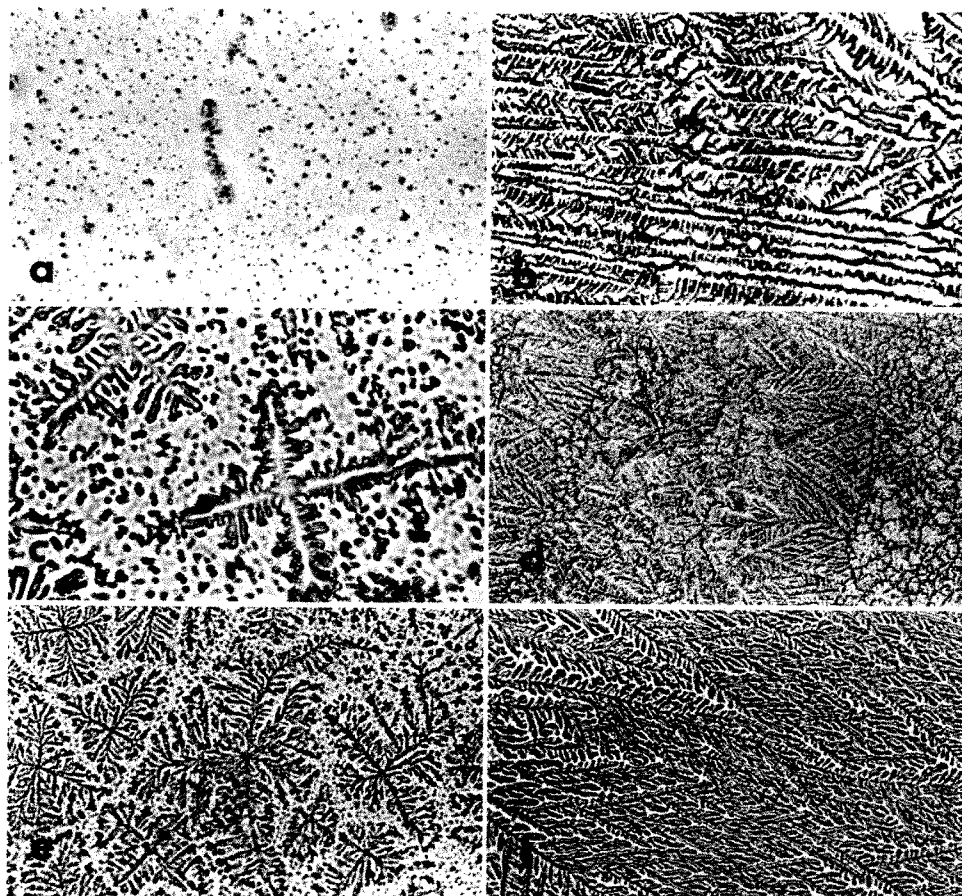
the pregnancy. Before distressing the patient with such weighty decisions, one has a positive obligation to confirm that the integrity of the amniotic sac has indeed been breached.

Following Papanicolaou's original observation of crystallization (arborization) of dried cervical mucus, this phenomenon was reproduced in most body fluids. Its occurrence is related to the presence of proteins and electrolytes (sodium chloride in particular) in the solution being investigated. Arborization has also been demonstrated in amniotic fluid. It is believed to reflect the relative concentrations of solutes. This characteristic has been used to differentiate amniotic fluid from urine and thus help make a diagnosis of ruptured membranes.<sup>1</sup>

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**Fig. 1.** Ferning of amniotic fluid at various gestational ages, air-drying (*left*) versus flame-drying (*right*): *a* and *b*, 6½ weeks; *c* and *d*, 12 weeks; *e* and *f*, 16 weeks. In air-dried specimens, the degree of arborization increases with gestational age with no ferning seen at 6½ weeks (*a*), early ferning at 12 weeks (*c*) and fully developed ferning at 16 weeks (*e*). All flame-dried specimens of fluid showed unmistakable arborization from 6½ weeks (*b*) to 16 weeks (*f*). (Contrast phase photomicrographs.  $\times 16$ ; except *c*,  $\times 40$ .)

Elias et al.<sup>2</sup> reported crystallization of amniotic fluid in 24 of 25 samples obtained at 16 to 18 weeks of gestation. We sought to evaluate if arborization of amniotic fluid is present in gestations of <16 weeks' duration. For this purpose, we obtained amniotic fluid from 10 patients in early pregnancy. The earliest amniotic fluid sample was obtained from an unruptured tubal gestation of approximately 6½ weeks' duration (46 days from last normal menstrual period). Specimens from gestations ranging from 8 to 14 weeks in duration were procured by aspiration at the time of pregnancy interruption. Amniotic fluid corresponding to the fifteenth and sixteenth weeks of pregnancy was sampled at the time of genetic amniocentesis.

The amniotic fluid specimen was spread over two glass slides. One specimen was smeared in a thin layer and allowed to air-dry before being examined microscopically under medium-power magnification ( $\times 16$ ). The second specimen was dripped onto a slide and flame-dried (but not boiled); it was then evaluated microscopically, as before. Crystallization (ferning) was

seen in all specimens that were rapidly heat-dried (Fig. 1, *b*, *d*, and *f*). When amniotic fluid was allowed to air-dry spontaneously, however, the appearance of arborization was inconsistent. There was a gradation seen with advancing gestation. None was seen in the 6½-week fluid (Fig. 1, *a*). There were a few clusters of ferning first seen at 8 weeks; this was somewhat more fully developed by 12 weeks (Fig. 1, *c*). Abundant ferning appeared in all second-trimester specimens (Fig. 1, *e*).

Failure of amniotic fluid from early pregnancy to arborize when allowed to air-dry may be misleading. Crystallization of the same specimen when flame-dried suggests that a thicker layer of fluid containing more proteins and electrolytes allows this phenomenon to occur. Air-drying requires a relatively thin layer of amniotic fluid. The absence of ferning may, therefore, result from the smaller absolute amount of solutes present. When thick drops of amniotic fluid were allowed to air-dry (a long, tedious process), comparable ferning did occur.

Ferning of amniotic fluid obtained at 6½ weeks of gestation suggests that crystallization is a property of amniotic fluid throughout pregnancy. Its failure to appear in some specimens may be an artifact relating to the preparation method. It is, therefore, recommended that a drop of the solution to be examined should be flame-dried for evaluation of ferning.

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## The prenatal diagnosis of Robin anomalad

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The Robin anomalad was diagnosed by the sonographic detection of polyhydramnios and fetal micrognathia in a patient at risk because of a previously affected child. Ultrasound in the second trimester failed to demonstrate any facial anomaly, but mandibular hypoplasia was clearly documented in the third trimester. The antenatal diagnosis allowed immediate neonatal assistance to prevent glossoptosis-induced respiratory failure. (*AM J OBSTET GYNECOL* 1986;154:630-2.)

**Key words:** Robin anomalad, prenatal diagnosis, ultrasound, fetus

The Robin anomalad is characterized by the association of micrognathia and glossoptosis. It is frequently accompanied by deformity of the palate, which may take the form of a postalveolar cleft or a high, arched palate.<sup>1</sup> The Robin anomalad is a neonatal emergency because the tongue may obstruct the airways and lead to suffocation. Antenatal recognition would permit the neonatologist to be present in the delivery room and to provide immediate care to the infant. The purpose of this communication is to report the prenatal diagnosis of the Robin anomalad.

#### Case report

The patient, a 31-year-old white woman, gravida 2, para 1, was referred for sonographic examination at 23 weeks of gestation because of a previous child affected with the Robin anomalad. The infant had required respiratory assistance and tube feeding at birth, and operation was performed for correction of a posterior cleft palate. Sonography demonstrated a singleton infant whose biometric parameters were consistent

with assigned gestational age (biparietal diameter, 5.7 cm; femur length, 3.9 cm; humerus, 3.7 cm; outer orbital dimension, 3.7 cm; tibial length, 3.4 cm; ulna, 3.4 cm). A careful examination did not reveal any visceral abnormalities. A midsagittal scan of the fetal face was obtained and is shown in Fig. 1. This scan was interpreted as normal. Coronal scans of the face demonstrated the integrity of the anterior maxilla (Fig. 2). As the accuracy of ultrasound in the detection of the Robin anomalad had not been tested, the patient was told that the sonogram was normal but the sonologist could not exclude the presence of such an anomaly. Another scan was obtained at 35 weeks. At that time polyhydramnios was noted and a midsagittal scan showed striking micrognathia (Fig. 3). The polyhydramnios worsened in the following 3 weeks. The patient went into spontaneous labor and was delivered of a female infant weighing 3600 gm with the typical features of the Robin anomalad, including a postalveolar cleft of the palate (Fig. 4). The infant required intubation in the delivery room; subsequently tube feeding was necessary. The neonatal course was otherwise unremarkable, and the infant will undergo surgical repair of the posterior cleft palate.

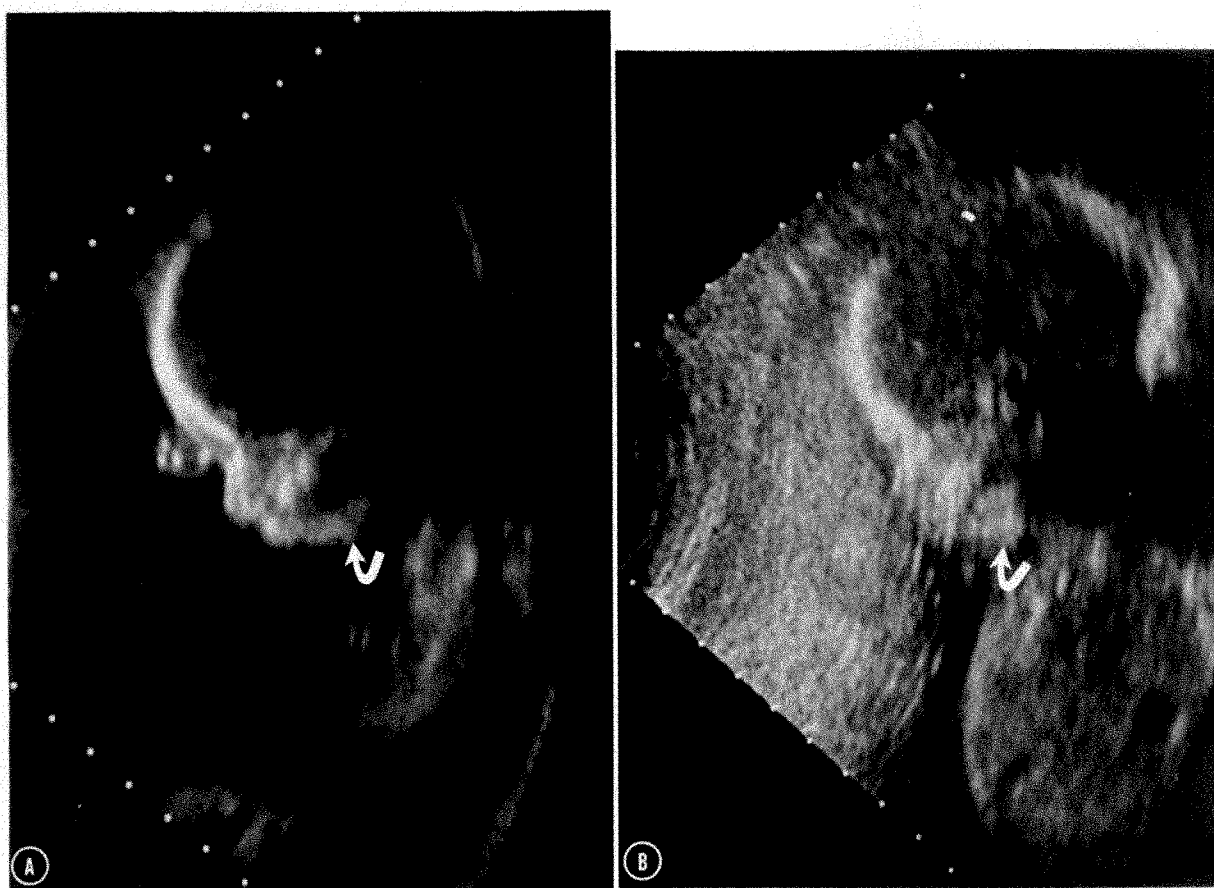
#### Comment

Recent advances in ultrasound imaging have permitted examination of the fetal face and the antenatal

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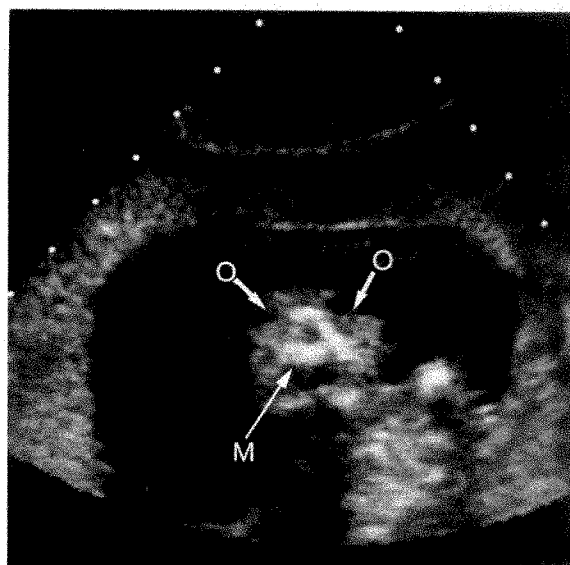


**Fig. 1.** A, Midsagittal scan of the face of a normal infant at 22 weeks of gestation. The arrow indicates the mandible. B, Midsagittal scan of the face of the index case at 23 weeks. The arrow points to a seemingly normal mandible.

recognition of facial dysmorphism.<sup>3</sup> Our case illustrates the feasibility of the prenatal identification of micrognathia. It is of interest that the sonographic examination performed in the second trimester failed to document such findings. This could be attributed to a primary diagnostic error of ultrasound. The natural history of the Robin anomalad before birth is unknown. Our case suggests micrognathia may develop in the latter part of gestation. If this were the case, an early prenatal diagnosis would have important limitations.

Although the diagnosis of cleft palate has been previously reported, it should be stressed that in all these cases the defect was anterior. The posterior palate is difficult to image with ultrasound; thus prenatal diagnosis of a defect located in this area is unlikely.

The association between polyhydramnios and the Robin anomalad has not been described. A pathogenetic explanation for this finding could be failure to swallow as a consequence of glossoptosis. Therefore, the sonographer should include the Robin anomalad in the differential diagnosis of polyhydramnios associated with fetal micrognathia.



**Fig. 2.** Coronal scan of the face in the index case at 23 weeks demonstrating the integrity of the anterior maxilla (M) (orbits = O).





**Fig. 3.** Midsagittal scan of the face in the index case at 35 weeks. Micrognathia is evident (arrow).

In our case, the diagnosis was made in a patient with a family history of the anomaly. Although the Robin anomalad is sporadic in most cases, both an autosomal recessive trait and an autosomal dominant trait with variable penetrance have been suggested. It is unclear to which of these our case can be attributed since no other affected relatives could be documented. Associated anomalies, especially congenital heart disease, are frequently found in the Robin anomalad.<sup>1</sup> Therefore,



**Fig. 4.** Photograph of the newborn infant showing features that confirm the diagnosis.

if this condition is suspected in a fetus, a thorough evaluation of fetal anatomy, including fetal echocardiography, is recommended.

We wish to acknowledge the editorial assistance of Mrs. Barbara Coster.

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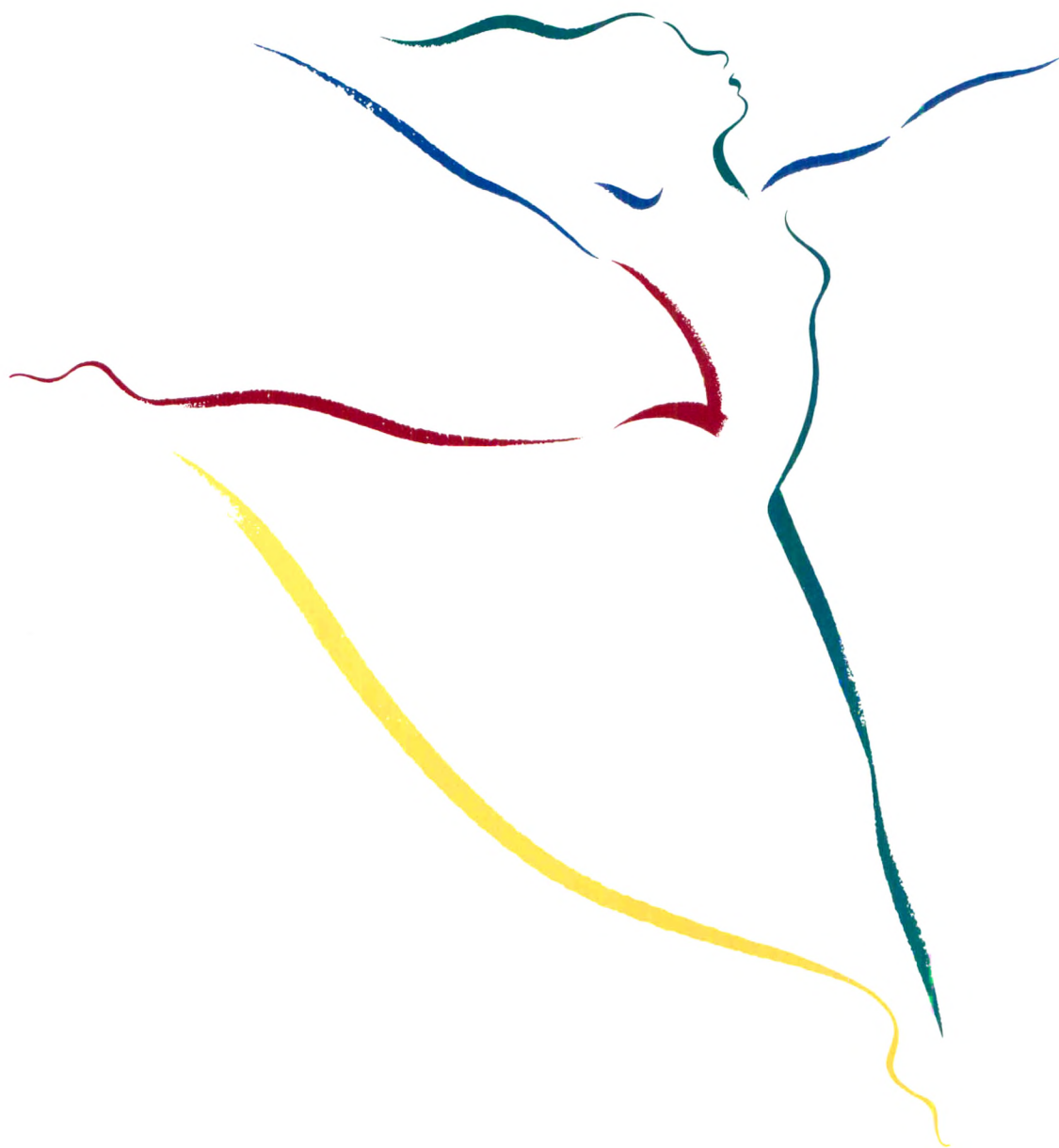
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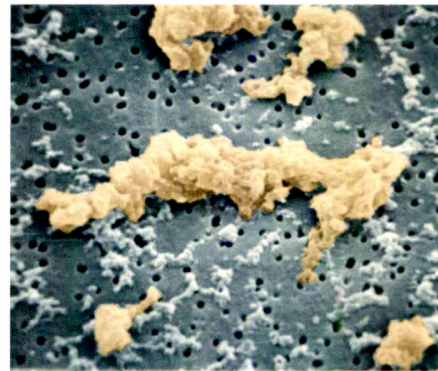
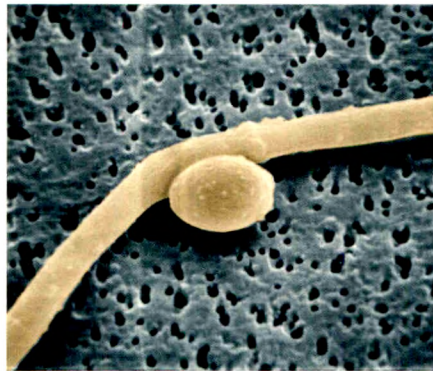
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December 1985



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# Postoperative intussusception after vaginal vault suspension

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Postoperative intussusception in the adult is a rarely reported and poorly understood condition. Presented is the case of a patient developing incomplete small bowel obstruction after an uneventful vaginal vault suspension. Jejunojejunal intussusception, requiring resection, was found at celiotomy. Intussusception must be considered in treating patients developing postoperative intestinal obstruction. (AM J OBSTET GYNECOL 1986;154:633-4.)

**Key words:** Postoperative intussusception, vaginal vault suspension

Postoperative intussusception is an infrequent but well-recognized complication of surgical procedures performed in children. Its occurrence in adults, however, is a largely unrecognized, and rarely reported, cause of small bowel obstruction.<sup>1</sup>

We report the case of a patient with postoperative intussusception after an uneventful Bailey-Williamson vaginal vault suspension. The pathophysiologic characteristics, diagnosis, and treatment of this uncommon condition are briefly discussed.

## Case report

A 60-year-old woman, para 4-0-1-4, had undergone vaginal hysterectomy and anterior repair 12 years previously. She had a 7-year history of total vaginal prolapse, third-degree cystocele, and second-degree rectocele. A combined procedure consisting of transabdominal Bailey-Williamson vaginal vault suspension and transvaginal cystocele and rectocele repairs was performed. After operation, although oral intake was generally well tolerated, the patient experienced intermittent nausea and vomiting. She was discharged on the seventh postoperative day.

Five days later the patient presented to the emergency department complaining of episodic nausea and bilious vomiting since discharge and dull epigastric and substernal pain for 1 day. She reported regular bowel function, with loose stools. Normally active bowel sounds, mild epigastric tenderness, and guaiac-negative stool were noted on physical examination. Serum chemistry analysis revealed a hypokalemic, hypochloremic metabolic alkalosis and dehydration. New T-wave inversions in the precordial leads were noted on the electrocardiogram. The patient was admitted, received nothing orally, and was hydrated. Abdominal roent-

genograms demonstrated an incomplete small bowel obstruction. After decompression by nasogastric tube and correction of fluid and electrolyte abnormalities, the substernal pain resolved and the electrocardiogram reverted to normal. Cardiac isoenzymes were negative. After 5 days of conservative treatment without resolution, the patient underwent reexploration for presumed small bowel obstruction secondary to adhesive bands. At celiotomy, an antegrade proximal jejunojejunal intussusception, 10 cm in length, was found. Attempts at manual reduction failed, necessitating resection. Pathologic examination of the specimen revealed submucosal venous congestion, mild acute inflammation and serosal adhesions. No other abnormality was noted.

After operation, the patient did well, was discharged on the sixth day, and was tolerating a regular diet.

## Comment

In the adult, intussusception is rarely recognized as a cause of small bowel obstruction in the postoperative period. Before 1980, several large series on postoperative small bowel obstruction failed to identify intussusception as a distinct entity. In 1981, however, Sarr et al.,<sup>1</sup> in a review of all adults with intussusception seen at the Mayo Clinic during a 23-year period, observed that intussusception was an important cause of postoperative intestinal obstruction. They found that intussusception occurred after abdominal operations for diverse conditions and that the diagnosis was made preoperatively only once in spite of contrast studies that had been obtained in many cases. Furthermore, most intussusceptions (17 of 22) occurred in the proximal jejunum. Before this report, postoperative intussusception had not been reported after gynecologic operations.

Postoperative intussusception represents a clinical entity that is different from the usual intussusception presenting de novo in the adult. A pathologic lesion, acting as a leading edge, is not found in postoperative intussusception. Symptoms of small intestinal obstruction usually develop within 2 weeks of operation, often

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after resumption of apparently normal bowel function. Obstruction, often insidious initially, is heralded by intermittent nausea, vomiting, and crampy abdominal pain. Conservative treatment is frequently protracted, especially if, as in the case presented, obstruction is incomplete, before subsequent reexploration is performed.<sup>1</sup>

The pathogenesis of postoperative intussusception remains unclear. The presence of intestinal suture lines, the use of long intestinal tubes, local adhesions, and altered peristalsis that is due to operative trauma have been implicated as causative factors.<sup>1</sup> In addition, extensive retroperitoneal dissection, chemotherapy, and radiotherapy have been postulated as causative agents in children.<sup>2</sup> In our patient, as in the majority of patients reported by Sarr et al.,<sup>1</sup> local adhesions, intimately associated with the intussuscepted segment, appear to be responsible.

Manual reduction of the intussusception should be

attempted in all cases. Less than 5% of postoperative intussusceptions in children require resection. In adults, however, approximately 60% of intussusceptions are not reducible, necessitating, as was the case in our patient, resection of the involved bowel. Strangulation is unusual. Successful reduction and lysis of surrounding adhesions eliminate the need for resection, since no recurrences have been reported.<sup>1</sup>

In conclusion, when a normal postoperative course is complicated by early small bowel obstruction or when a prolonged "ileus" is encountered, intussusception must be considered as a possible cause.

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## Leg static pressure values in pregnant women with iatrogenic pulmonary edema

Robert C. Goodlin, M.D.

Omaha, Nebraska, and Denver, Colorado

In two pregnant women with iatrogenic pulmonary edema, impedance plethysmographic measurements indicated low values for maternal cardiac output and venous resistance (or static pressure). Decreased venous tone could account for the pulmonary edema. (*AM J OBSTET GYNECOL* 1986;154:634-5.)

**Key words:** Leg static pressure, iatrogenic pulmonary edema, impedance plethysmography

The laboratory measure of static pressure is important in assessing the ratio of the blood volume to the vascular capacity of the animal. Static pressure is a determinant of cardiac output and is elevated in experimental hypertension, in normal pregnant animals, and in humans in heart failure. It is usually determined in laboratory animals by producing acute cardiac arrest and recording the pressure when the venous and arterial sides are equal. We have used a modification of the demonstration that static pressure divided by "re-

sistance of venous return" determines cardiac output ( $CO = SP/RVR$ ). Noninvasive plethysmographic techniques are capable of estimating both cardiac output and venous resistance, allowing an estimate of leg static pressure in otherwise undisturbed pregnant women ( $SP = CO \times RVR$ ).<sup>1</sup> During the course of evaluation of leg static pressure determinations in pregnant women, two pregnant subjects with iatrogenic pulmonary edema were also studied.

Noninvasive impedance plethysmographic techniques for cardiac output and venous resistance were performed according to techniques previously described.<sup>1</sup>

#### Case reports

**Case 1.** An 18-year-old white woman, para 0-0-0-0, with severe hypertension (160/110 torr), elevated liver enzymes (serum oxaloacetic transaminase =

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280 units), and thrombocytopenia (platelet count = 68,000; plasma volume = 36 ml/kg, normal  $65 \pm 6$  ml/kg) was treated initially with steroids, plasma volume expansion, and antihypertensive drugs (hydralazine) in her twenty-fifth week of pregnancy. By the third day of hospitalization, there was a significant fall in hematocrit (41% to 28%) and a loud systolic pulmonic flow murmur developed. Chest x-ray film showed pulmonary edema with "normal heart size." Measurement of cardiac output with a Swan-Ganz thermodilution technique indicated during 4 hours' time an average cardiac index of 2.8 and cardiac performance was normal. The resistance to venous return was 0.03 (normal 0.9), giving a calculated estimate of leg static pressure of "3.0" (normal "10"). The patient was treated with furosemide, 25 mg intravenously, had a profound diuresis, and 48 hours later had a rise in hematocrit to 35%. Blood pressure remained at near-normal levels, and with the onset of labor she was delivered in the twenty-sixth week by cesarean section of a 780 gm infant in the breech position. Maternal ascites was noted at the time of delivery.

**Case 2.** A 25-year-old woman, para 0-0-0-0, with a twin pregnancy at 31 weeks had a plasma volume of 51 ml/kg (normal for twins 78 ml/kg). She was admitted in premature labor and was treated initially with 25 gm of albumin intravenously, steroids, and terbutaline to inhibit labor. On the third hospital day, she became dyspneic and had a loud pulmonic systolic flow murmur. Chest x-ray film showed pulmonary edema with "normal heart size." Cardiac index with the impedance phlebograph was 2.3 and the resistance to venous return was 0.03. Calculated estimate of leg static pressure was "2.8" (normal "10"). She was treated with a single injection of 25 mg of furosemide intravenously, had a profound diuresis, and dyspnea disappeared. Hematocrit rose from 27% to 35% and leg static pressure rose to "15" during 2 days' time. She remained at bed rest. Because one fetus showed evidence of growth retardation, labor was induced at 35 weeks when the amniotic fluid indicated a mature lecithin/sphingomyelin ratio. Both she and her infants did well.

#### Comment

In 1940, Starr defined the term "static pressure" as that pressure existing at all points in the circulation

when the heart is stopped and suggested that it was increased in heart failure because of increased plasma volume.<sup>2</sup> The increase in plasma volume seen during normal pregnancy should both increase static pressure and at the same time reduce resistance to venous return, resulting in a marked increase in cardiac output. When maternal cardiac output is below normal, despite a markedly reduced "resistance to venous return," static pressure should then be very low. This was the apparent situation in both of these cases, perhaps reflecting a markedly dilated venous system in the calf with subsequent reduced venous tone. If generalized throughout the body, such venous dilation would lead to marked plethora. Ascites and pulmonary edema could also reflect the reduced colloid osmotic pressure associated with the rapid expansion of plasma volume, as was indicated by declining hematocrits in both of these cases. The basic problem may be that of failure of the overdilated venous system to return blood to the heart and of reduced glomerular filtration. Appropriate therapy would be to reduce plasma volume, increase renal function, and/or increase venous tone (resistance to venous return). However, both of these patients responded to furosemide, which, in addition to being a powerful diuretic, is considered to increase venous capacitance.<sup>1</sup>

Pregnancy well-being is associated with a significant increase in venous capacitance and plasma volume. The tocolytic drugs may exaggerate this effect, reducing venous tone and resistance to venous return to the point of pulmonary edema and generalized plethora.

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# Prenatal diagnosis of fetal renal mesoblastic nephroma

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A rare case of fetal renal mesoblastic nephroma diagnosed prenatally by ultrasonography is presented.  
(AM J OBSTET GYNECOL 1986;154:636-7.)

The use of ultrasound for the assessment of congenital anomalies of the fetus when polyhydramnios is present is well established. In this report, a case of fetal mesoblastic nephroma diagnosed prenatally at 27 weeks of gestation is presented. To our knowledge, this entity has been diagnosed on only one other occasion.<sup>1</sup>

## Case report

A 26-year-old, gravida 4-2-0-1 woman presented at 26 weeks of gestation in premature labor. The uterus was much larger than expected and measured 42 cm from the top of the symphysis pubis to the uterine fundus. An ultrasound scan was ordered. Sonography revealed a single fetus in a breech presentation with fetal age compatible with 27 weeks and with gross polyhydramnios. Scans of the fetal abdomen revealed a solid tumor in the region of the left kidney (Fig. 1). The left kidney was approximately three times the size of the right kidney, which appeared normal. The fetal abdominal circumference was 25.1 cm and the tumor circumference 10.4 cm, resulting in a ratio of 0.41. The patient was treated with intravenous ritodrine to stop labor, but the therapy failed; because of breech presentation, she was delivered by cesarean section of a 1100 gm baby. The baby did well and did not develop respiratory distress syndrome. Following stabilization and evaluation of the baby, a left nephrectomy was performed. The pathologic specimen revealed a renal mesoblastic nephroma. The baby did well postoperatively.

## Comment

Fetal renal tumors are rare, and this case of mesoblastic nephroma has been reported on only one occasion, by Giulian.<sup>1</sup> The incidence of all renal tumors is 7.8 per million children under the age of 15, with Wilms' tumor being most common. Renal mesoblastic nephroma also has several other names such as

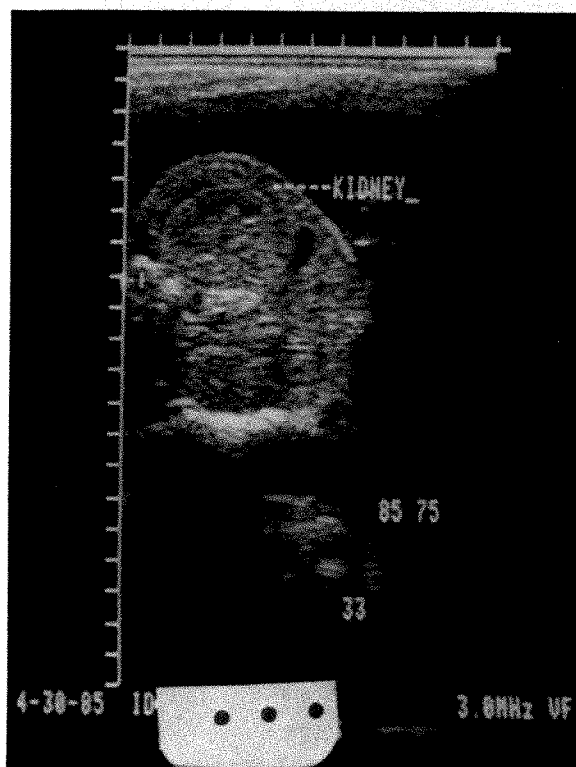


Fig. 1. Cross section of fetal abdomen revealing renal tumor. Fetal spine is to the left.

leiomyomatous hamartoma, fetal renal hamartoma, congenital mesoblastic nephroma, and mesenchymal hamartoma.

The ultrasonic findings of a mesonephric nephroma are similar to the appearance of Wilms' tumor, which also is predominantly a solid tumor. Wilms' tumor and mesoblastic nephroma may not be distinguishable sonographically. Hartman et al.<sup>2</sup> studied neonates with mesoblastic nephromas and found that most of the tumors had solid sonographic features of low-level echoes within the tumor. Our case also had echoes within the tumor as well as a well-formed capsule around the tumor. This capsule probably represents the interface between the tumor and the normal parenchyma. Most mesoblastic nephromas are unilateral and present as an enlargement of the involved kidney and bulging of

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the abdomen. The case reported by Giulian involved the right kidney, and our case involved the left kidney. The association of fetal renal tumors and polyhydramnios has been noted.

Histologic features of the tumor include a preponderance of spindle cells resembling fibroblasts and smooth muscle cells. Pleomorphism and mitotic figures are common. The usual treatment for mesonephric nephromas is unilateral nephrectomy without irradiation

or chemotherapy. Most are benign tumors, but occasionally they may recur.

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## Perforated adenocarcinoma of the appendix during pregnancy

Alan E. Donnenfeld, M.D., Nancy S. Roberts, M.D., Thomas A. Losure, D.O., and Arthur W. Mellen, M.D.

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Ruptured appendicitis was suspected in a primigravid woman at 31 weeks' gestation. Invasive adenocarcinoma of the appendix was diagnosed pathologically. To our knowledge, this is the first reported case during pregnancy. (*Am J Obstet Gynecol* 1986;154:637-8.)

**Key words:** Adenocarcinoma of the appendix, appendicitis, hemicolectomy

Primary adenocarcinoma of the appendix is found in between one in 5000 and one in 30,000 appendectomy specimens examined histopathologically. Less than 200 total cases have been reported in the world literature and only six cases in patients <30 years of age. To our knowledge, no cases have been reported during pregnancy. Timing of delivery and the need for further surgical intervention created extremely difficult management decisions in attempting to maximize care for both mother and fetus.

#### Case report

A 25-year-old white primigravid woman at 31 weeks' gestation presented, complaining of 8 days of diffuse lower abdominal and back pain with increasing severity in abdominal discomfort over the past 48 hours. The pain was most severe in the right lower quadrant and was aggravated by fetal movement. The patient reported night sweats, shaking chills, nausea, and vomiting over the past 24 hours. She was pale and diaphoretic, and she writhed with abdominal pain on fetal movement. Blood pressure was 110/70 mm Hg and temperature was 103° F. Abdominal examination revealed markedly decreased bowel sounds with diffuse

tenderness. Guarding and rebound were most prominent in the right lower quadrant. Uterine fundal size was consistent with dates. White blood cell count was 9000/ml with 75% polymorphonuclear cells, and hematocrit was 32%. External fetal monitoring revealed a reactive nonstress test with a baseline of 180 bpm. Contraction frequency was every 4 minutes. Ultrasonography demonstrated an appropriate for gestational age fetus in cephalic presentation. Amniotic fluid was normal.

Intravenous ritodrine therapy at 0.35 mg/min was begun, and a decision was made to proceed to laparotomy because of peritonitis and probable appendicitis. A ruptured appendix encased in dense adhesions within a 4 by 5 cm abscess cavity was encountered. The appendiceal stump was excised, the abscess cavity drained, and the peritoneal cavity lavaged with normal saline solution. Administration of intravenous ampicillin, tobramycin, and clindamycin was started.

On the fifth postoperative day, pathologic identification of deeply invasive grade 1 mucinous adenocarcinoma of the appendix causing perforation, with secondary acute appendicitis, was documented. Oxytocin induction resulting in vaginal delivery of a healthy 2160 gm female infant was accomplished at 33 weeks' gestation. The baby appeared completely normal. Pathologic examination of the placenta was also normal. Three days post partum, a chest x-ray film, liver-spleen scan, and colonoscopy revealed no evidence of metastases. Abdominal computerized tomographic scan showed a small right lower-quadrant fluid collection and an intravenous pyelogram demonstrated mild

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bilateral hydroureters consistent with recent pregnancy. Right hemicolectomy and omentectomy were performed 9 days post partum. Many large, inflammatory, mesenteric lymph nodes were palpated. No evidence of metastatic disease was identifiable grossly or histopathologically. Nineteen mesenteric lymph nodes were identified, all free of tumor cells. The patient was discharged to her home after an uneventful postoperative course, 32 days after appendectomy and 19 days after delivery with a thriving baby girl.

#### Comment

The pregnant state, because of altered immunologic tolerance to foreign, paternally derived antigens, was once believed to be more susceptible to malignancy. The maternal immunologic acceptance of the fetus, however, is specific. The incidence of malignancy during pregnancy, approximately one per 1000, is identical to age-matched nonpregnant women. Therefore pregnancy does not confer an alteration in a woman's oncogenic potential.

The placental barrier is an effective deterrent to fetal metastases, even in cases of widespread, aggressive malignancies. Potter and Schoeneman<sup>1</sup> found only 24 cases of maternal-to-fetal and placental cancer spread in the world literature. There were eight cases in which direct transplacental spread to the fetus occurred; seven were caused by melanoma and one by lymphosarcoma. Six of these infants died within 1 year of acquired metastatic disease.

The diagnosis of acute appendicitis during pregnancy is a difficult task. Misdiagnosis rates range from 41% to 52%. Compared to nonpregnant women in the same age range, however, the diagnostic error rate is surprisingly equivalent, ranging from 30% to 45%. The incidence of appendicitis during pregnancy is 10 to 20 per thousand.<sup>2</sup> These rates are identical to the incidence in the general population. Perforation of the appendix is much higher in pregnant than in nonpregnant women, 30% to 38% during pregnancy compared to 14% to 21% in the nonpregnant female, perhaps because the diagnosis is made at a later stage because of hesitation to perform laparotomy.

Five-year survival rates in patients with adenocarcinoma of the appendix are 20% in those treated by appendectomy alone compared to 63% in patients subjected to right hemicolectomy. Unfortunately, treatment recommendations for adenocarcinoma of the appendix have not detailed survival rates in those patients who have had appendiceal rupture and intraperitoneal spread. In addition, the effect of generalized peritonitis possibly reducing metastatic invasion is unknown.

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# Ovarian cancer in the elderly: An analysis of Surveillance, Epidemiology, and End Results Program data

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With use of a unique data set from the Surveillance, Epidemiology, and End Results Program of the National Cancer Institute for 11,062 women diagnosed with ovarian cancer from 1973 to 1982, this study focuses on the impact of old age on this malignancy. Aspects of ovarian cancer as it pertains especially to elderly women (those 65 years or older) are examined according to age/stage relationships at initial diagnosis and age variations in treatment and survival. Elderly women are more likely than younger women to be in advanced stages of ovarian cancer at initial diagnosis, and they constitute about 42% of this group. In the stage-unknown category, over 50% are 65 years or older. Data suggest that elderly women are treated more conservatively than younger patients. The National Cancer Institute data also illustrate the increased preference to treat this neoplasm with surgical procedures and chemotherapy rather than surgical procedures and radiation. For Stages III and IV disease, 5-year relative survival rates for elderly women are almost one half of the rate observed for women under 65. Although the prognosis of patients with advanced ovarian tumors is poor for all, it is even worse as age progresses. (AM J OBSTET GYNECOL 1986;154:639-47.)

**Key words:** Elderly, ovarian cancer, age contrasts, survival, stage/age relationships

Of all gynecologic neoplasms, ovarian cancer is the least responsive to all therapeutic modalities. Only 37% of all ovarian cancer patients survive for 5 years following the diagnosis of this malignancy. There has been little change in the survival rate over the last 15 years.<sup>1</sup> In 1985 the disease will afflict approximately 18,500 women and, as one of the leading causes of female cancer deaths, will claim the lives of 11,600 women.<sup>2,3</sup>

For elderly women, ovarian cancer appears to be particularly severe. Data from the Surveillance, Epidemiology, and End Results (SEER) Program of the National Cancer Institute show that peak incidence rates are observed in women between the ages of 60 and 85 years, with a disproportionate occurrence of advanced disease found in the age segment of women 65 years or older. Also, more than 42% of those who present with Stages III and IV ovarian cancer at initial diagnosis are older-aged women.

This paper uses data of the SEER Program for 11,062

women of all races diagnosed with ovarian cancer during 1973 to 1982 to show that age should be considered with other factors as to how it influences the diagnosis, treatment, and prognosis of ovarian cancer. The purpose of this report is to document and highlight aspects of ovarian cancer as it pertains especially to elderly women. Any advances in our ability to understand the trajectory of this malignancy could provide clues to improve early detection and better patient management.

Surprisingly, there is not much clinical literature relevant to ovarian cancer treatment and response to therapy in women 65 years or older. When age factors are presented, the discussion is usually limited to describing the age range or the median age of the clinical trials study patients. Table I shows age distributions in selected studies.

Patients described in ovarian cancer clinical studies tend to have median or mean ages of from 52 to 58 years.<sup>4-12</sup> This is younger than the ovarian cancer patient population reflected in the data of the SEER Program, which has a median age of 61 years across stages. Specific age groupings are frequently not identified beyond the general menopausal distinction (for example, under 50 years and 50 years or older)<sup>13,14</sup> or wide age

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**Table I.** Distribution by age of ovarian cancer patients in selected studies

Reference	No. of patients	Age range (yr)	Median age (yr)	Mean age (yr)
Bast et al., 1983 <sup>4</sup>	101		54	
Dembo et al., 1979 <sup>13</sup>	231	<50; >50		
Demopoulos et al., 1984 <sup>15</sup>	241	<40 - >70		
De Palo et al., 1981 <sup>16</sup>	51	21 - 74		
Free and Bourne, 1982 <sup>17</sup>	188	16 - 85		
Greco et al., 1981 <sup>9</sup>	59	36 - 79		52
Guthrie et al., 1984 <sup>5</sup>	656	18 - 95	54	
Hreshchysyn et al., 1980 <sup>22</sup>	86*			
Katsube et al., 1982 <sup>18</sup>	371	<20 - >60		
Kennedy and Gordon, 1981 <sup>10</sup>	97	11 - 83		58
Khan et al., 1983 <sup>11</sup>	85	29 - 75		55
Ozols et al., 1979 <sup>23</sup>	82*			
Piver et al., 1976 <sup>6</sup>	100	30 - 81	53	
Piver et al., 1984 <sup>7</sup>	201	15 - 80 +	53	
Pohl et al., 1984 <sup>12</sup>	172	16 - 87		57.4
Sigurdsson et al., 1983 <sup>24</sup>	494*			
Smith and Day, 1979 <sup>19</sup>	1903	40 - 70		
Tolino et al., 1981 <sup>20</sup>	22	24 - 73		
Weekes and Watkins, 1981 <sup>21</sup>	50	20 - 90		
Wharton et al., 1984 <sup>14</sup>	395	≤45 - >45		
Young et al., 1983 <sup>8</sup>	143	15 - 79	52	

\*No age mentioned.

ranges are given<sup>15-21</sup> in the clinical literature. Indeed the numbers of patients reported in most treatment series are small, so it would be difficult to analyze data according to several age breakdowns. Sometimes, even with large numbers of patients reviewed, age may not be mentioned.<sup>22-24</sup>

The value of using the large information base of the SEER Program, which provides treatment data on ovarian cancer patients in a defined population, is that it describes the patterns of care and survival over time according to age variations. Although limited in clinical detail, these data may provide leads to help identify secular changes in stage and survival representative of the ovarian cancer population. The emphasis on the effects produced in the different age categories can promote development of hypotheses and specific studies related to diagnosis, staging, treatment, and survival from ovarian cancer.

Following a brief discussion of the incidence of ovarian cancer according to age, variations in extent of disease by stage according to age at initial diagnosis are described. Age is then cast against all types of treatment given. Following this, a year-by-year portrayal of the overall treatment trends from 1973 to 1982 is presented, after which the survival patterns according to specific age groups are compared.

#### Material and methods

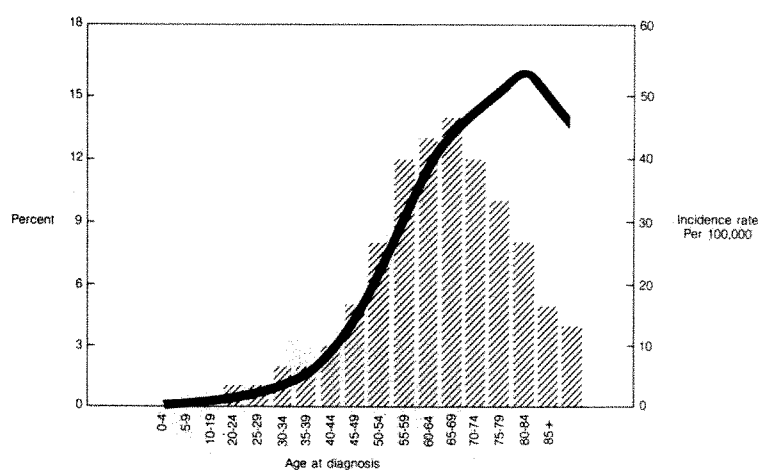
Data for this report consist of information obtained from the SEER Program registries of the states of Connecticut, Iowa, New Mexico, Utah, and Hawaii, as well as the reporting regions of Detroit, Atlanta, and Seattle. The large-scale population-based SEER Program data system represents about 10% of the population of the

United States. The participants in the SEER Program and a complete description of the program have been published in several documents.<sup>1-3</sup>

Demographic data and disease-specific information including primary site, histologic type, extent of disease, treatment, and follow-up are available from the SEER Program. Unless specified otherwise, the discussion that follows refers to age variations associated with the treatment of ovarian cancer in 11,062 patients of all races and all histologic types in combination. Regarding histologic types, most tumors (93%) are epithelial in origin with the largest number of cases classified as adenocarcinoma, not otherwise specified; papillary adenocarcinoma, not otherwise specified; and papillary serous cystadenocarcinoma. Almost 6% are classified as carcinoma, not otherwise specified. Only 1% are malignant teratomas. This histologic classification conforms with other reports in which the pathologic conditions of ovarian cancer are discussed.<sup>18, 24, 25</sup>

For all analyses in this paper, the age breakdown is <45 years, 45 to 54, 55 to 64, 65 to 74, and ≥75. Elderly women are defined in the study as those in the two uppermost age groups of 65 to 74 and 75 years and older.

This descriptive analysis addresses whether elderly women differ according to diagnosis and treatment from younger or middle-aged women and whether their survival experience varies. Specific questions for each category are as follows: (1) Diagnosis. Are older women more likely to present with advanced stage disease at initial diagnosis? (2) Treatment. Do older women receive the same type of treatment as younger or middle-aged women within Stages I and II (early ovarian cancer) and Stages III and IV (advanced ovar-



Source: NCI SEER Program, N = 11,062

Fig. 1. Incidence rates by age, 1973 to 1982.

ian cancer)? (3) Survival. What is the survival experience of elderly women who have been diagnosed as having ovarian cancer compared to that of younger and middle-aged women?

The first two questions were answered with use of the age variable and the SEER Program coded staging and treatment variables. Response to the third question used SEER Program survival rates, which are based on first primary cases diagnosed from 1973 through 1980 and are presented as 5-year relative survival rates.

Statistical methods used to assess differences among the age groups are the standard nonparametric techniques for categorical variables and contingency table analyses. Survival tables are generated by the life table method. The relative survival rate is the ratio of the observed survival rate for patients with cancer to the expected survival rate in a standard population for persons according to age, sex, and race. Thus relative survival rate is an adjustment for deaths resulting from causes other than cancer.

The staging system used in the SEER Program is consistent with the one used by the International Federation of Gynecology and Obstetrics: Stage I, localized to ovarian tissue; Stage II, extension to the peritoneum, fallopian tube, or implants adjacent to the ovaries; Stage III, involvement of retroperitoneal lymph nodes or regional extension; Stage IV, distant metastases. However, SEER Program data have not distinguished retroperitoneal nodes from other nodes, and therefore Stages III and IV disease cannot be separated for these analyses.

Treatment regimens are classified as surgery; surgery in combination with radiation and/or chemotherapy; and a combination of surgery, radiation, and chemotherapy. For patients in Stages III and IV of ovarian cancer and when numbers permit, a regimen of either radiation or chemotherapy alone or a combination of both may also be included. Surgery is defined as the

first course of therapy received by the patient within the first 4 months of initiation of therapy; no exploratory or diagnostic procedures, including laparotomy without intent of treatment, are so classified. Methods and schedules for radiation and chemotherapy are not specified in the SEER Program system of reporting. All modalities initiated in the first 4 months are included regardless of sequence of completion. Chemotherapy initiated and given only in the physician's office is not reported.

Survival data are for 8336 ovarian cancer cases followed through 5 years. Relative survival rates, that is, rates adjusted to estimate survival from effects of cancer only, are used.

## Results

**Incidence rates.** Fig. 1 presents the age-adjusted incidence rates for all first primary ovarian cancers diagnosed during 1973 to 1982 in juxtaposition to the age distribution of the SEER Program ovarian cancer study population. As Fig. 1 shows, ovarian cancer occurs infrequently in women below 40 years. Beginning with the 40 to 44 age group, which has a rate of 15.7 per 100,000 cases, the incidence rates increase dramatically as age advances. The rate more than doubles after age 50 to about 35 per 100,000. Highest incidence rates are found in the age range from 65 to 85 with the peak rate of 54 per 100,000 found in the 75 to 79 age group.

The largest number of ovarian cancer patients is found within the 60 to 64 age group. Over one third of the SEER Program study population (38%) is 65 years or older.

**Median age according to stage.** The older the age, the later the stage. The median age observed for stage of ovarian cancer is 54 years for Stage I; 58 years for Stage II; and 62 years for Stages III and IV. For cases in which the stage is unknown, the median age is 65

**Table II.** Distribution according to age within stages of the disease for ovarian cancer patients at initial diagnosis\*

Stage	n†	Age distribution (%)					
		<45 yr	45-54 yr	55-64 yr	65-74 yr	≥75 yr	All ages‡
I	2916	26.9	24.1	24.2	14.5	10.3	100.0
II	317	14.5	25.6	30.9	18.6	10.4	100.0
III-IV	7309	10.6	18.1	28.9	24.4	17.9	100.0
Stage unknown	520	11.3	17.5	20.8	23.7	26.7	100.0

\*Source: National Cancer Institute SEER Program, 1973-1982.

†Total = 11,062 (excludes carcinoma in situ).

‡Percentages may not add to 100 because of rounding.

**Table III.** Stage distribution by age of ovarian cancer patients, 1973-1982\*

Age (yr)	n†	Stage distribution (%)				
		I	II	III and IV	Unknown	Total
<45	1668	47.1	2.8	46.6	3.5	100.0
45-54	2196	32.0	3.7	60.2	4.1	100.0
55-64	3028	23.3	3.2	69.8	3.6	100.0
65-74	2388	17.7	2.5	74.7	5.2	100.0
≥75	1782	16.8	1.9	73.5	7.8	100.0

\*Source: National Cancer Institute SEER Program.

†Total = 11,062.

years. Cases of elderly patients with cancer may go unstaged because of the limited probability of treatment success. The older median age for the stage-unknown group also suggests that elderly women may not be subjected to the procedures used for definitive staging (that is, laparotomies) or that clinical evidence indicated the disease was far advanced and diagnosis was possible by ascitic cytologic examination.

**Age-stage distribution.** The workup for ovarian cancer usually requires abdominal exploration to accurately stage the disease. Age-stage comparisons are presented in Table II. The percentages of the age groups are organized according to stage. Two thirds of the cases are classified as being in Stages III and IV.

Of women diagnosed with Stage I disease, more than 75% are younger than 65 years of age. For Stage II disease, 71% are younger than 65. Older women constitute about 42% of the group of patients with Stages III or IV ovarian cancer; in the stage-unknown classification, older women constitute slightly more than 50% of the group.

**Stage-age distribution.** Women 65 years or older are more likely than younger women to be in later stages of ovarian cancer at initial diagnosis. Table III shows the distribution of ovarian cancer patients in the study cohort according to stage of disease. Most of the advanced stage patients are older women. Approximately 75% of those 65 to 74 years of age have Stages III and IV disease; about 74% of those 75 and older are observed to be in the later stages also. By contrast, 47%

of the youngest age group, women less than 45 years, are initially diagnosed as being in Stage I of ovarian cancer. Younger age is associated with earlier stage disease. Older women are more likely to present at initial diagnosis with advanced stages of ovarian cancer.

#### Treatment

**Stages I and II.** Treatment data are available for 3194 SEER Program ovarian cancer patients with Stages I and II disease. Both stages indicate the neoplasm is confined within the pelvis. The data are collapsed for this analysis. Fig. 2 portrays observations for the dominant treatment modality groupings: surgery; surgery and radiation; surgery and chemotherapy; surgery, radiation, and chemotherapy. The bulk of all age groups are treated by surgical procedures alone or in combination with other modalities. The oldest age group, 75 years or older, receives more surgical procedures and a great deal less of the combined modalities. Overall, more surgical treatment alone and less in combination with radiation and/or chemotherapy is seen with progression of age.

Treatment for patients under 45 years varies from that for the older patient groups. Those under 45 years are treated more often by surgical procedures and chemotherapy, 21%, and less often by surgical procedures and radiation, 14%. These data demonstrate that as women advance in age, they are given combined therapeutic modalities less frequently.

**Stages III and IV.** Treatment patterns for women with advanced ovarian cancer in the different age groups

are described for 7309 patients. With this large number, it is possible to look at the additional treatment options of radiation and chemotherapy singly and in combination. Fig. 3 presents treatment data for Stages III and IV by age.

Although some controversy still exists as to the efficacy of adjuvant radiation or chemotherapy, clearly the treatment of choice for advanced ovarian cancer is the surgical procedure.<sup>26, 27</sup> Overall, more than two thirds of the SEER Program patients with Stages III and IV disease received surgical treatment either alone or in combination with radiation and/or chemotherapy.

However, a look within age groups reveals a finding similar to that just described for early-stage ovarian cancer. All the age groups underwent a surgical procedure alone as a treatment modality in about the same proportions, from slightly over 17% to almost 22%. The highest proportion, 21.7%, was observed in the 75 years and older age group. With advancing age, use of surgical procedures in combination with other modalities diminishes. Figures for the successive age groups show a decrease in the proportion of patients treated with combined modalities of surgery with radiation and surgery with chemotherapy.

More older women receive only chemotherapy or only radiotherapy. Of 1216 women receiving only chemotherapy, more than 45% were 65 years or older. For those receiving only radiotherapy (although a much smaller group of 93 women), more than half were 65 years or older. From these data, one may conclude that single modality therapy (surgery, radiation, or chemotherapy) is associated with advancing age.

A subset of patients with Stages III and IV ovarian cancer warrants some attention. More than 10% (784) of the group received no known therapy. The two oldest age groups account for 76% (597) of these patients. These data suggest that older women may be considered poor treatment risks.

**Stage unknown.** Approximately 5% (520) of the SEER Program ovarian cancer patients are in the stage-unknown category. Fig. 4 shows the same treatment combinations as previously displayed. Slightly more than 50% (262) are 65 years or older; about 22% of all ages received surgical treatment as a single agent, 15% of the total group received chemotherapy alone, and 15% received a combination of surgical procedure and chemotherapy. More of the older patients received the single modality of chemotherapy.

Close to 28% of the ovarian cancer patients with an unknown stage had no known treatment specified. Once again, a preponderance of elderly persons appear in this nebulous classification. Of the 144 individuals so classified, 76% (110) were 65 years or older.

**Treatment trends.** Use of chemotherapy in combination with surgical procedures has increased consid-

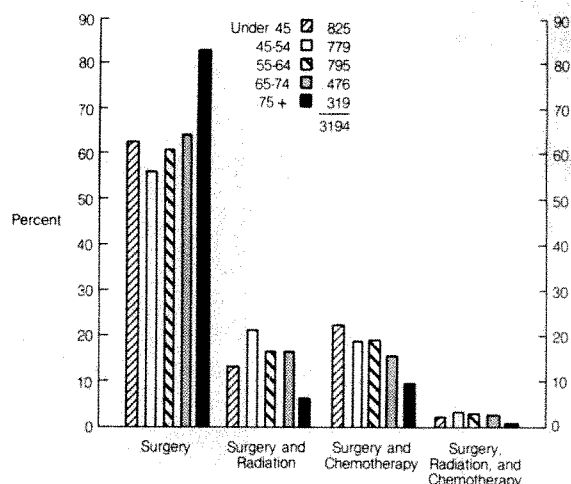


Fig. 2. Treatment distribution by age: Stages I and II.

erably compared to use of surgery with radiation therapy over the last decade. SEER Program data over a 10-year time span permit an examination of treatment trends. It is known from the clinical literature<sup>8, 26-27</sup> that there have been changes in the choice of treatment for ovarian cancer in recent years. The changes are dramatically illustrated in Fig. 5 with data from the SEER Program cohort for white ovarian cancer patients treated during 1973 to 1982 with a combination of surgical and radiation procedures or with the combination of surgical procedure and chemotherapy. A comparison of these two dominant therapy combinations over this 10-year period shows a decline in use of the combination of surgery with radiation from 21.3%, a peak in 1974, to a low of 3.3% in 1982. On the other hand, use of surgery with chemotherapy ranged from a low of 11.7% in 1973 to a maximum of 41.9% in 1982. This trend depicts what is generally described in the treatment literature. Chemotherapy now often follows surgical procedures for advanced ovarian cancer. In recent years the treatment of choice for ovarian cancer is either aggressive surgical procedure alone or in combination with chemotherapy.

The pattern of practice reflected here was largely influenced and altered by a randomized trial conducted at the M. D. Anderson Hospital in the early 1970s.<sup>28</sup> Radiation therapy for ovarian cancer is difficult because of (1) the spread throughout the abdomen early in the course of the cancer, (2) the dose-limiting sensitivity of intraabdominal organs, and (3) inability to direct the radiation to selected abdominal regions. Both the abdominal bath and moving-strip techniques have therapeutic limitations. Radiation therapy as postoperative therapy has been largely abandoned in cancer patients with Stages III and IV disease. Except for clearly defined subgroups of patients (Stages IB, II, and asymptomatic III presentations), radiation is not used.<sup>13, 27</sup>



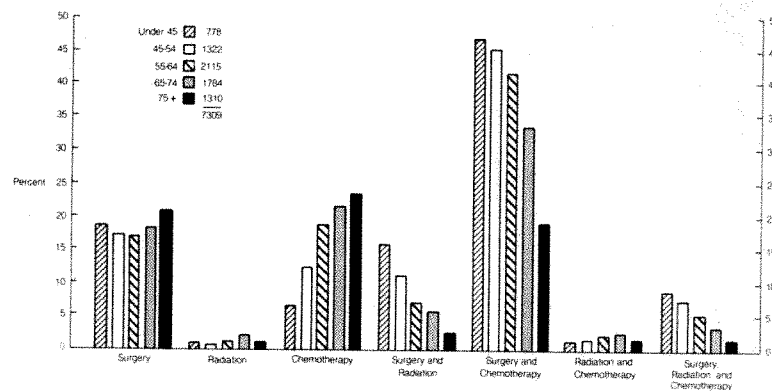


Fig. 3. Treatment distribution by age: Stages III and IV.

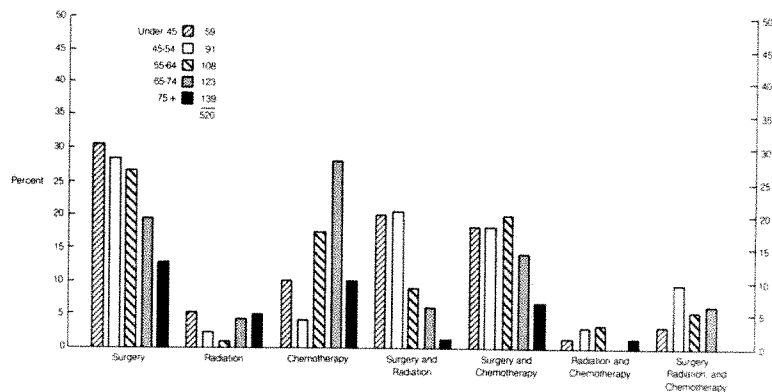


Fig. 4. Treatment distribution by age: stage unknown.

### Survival

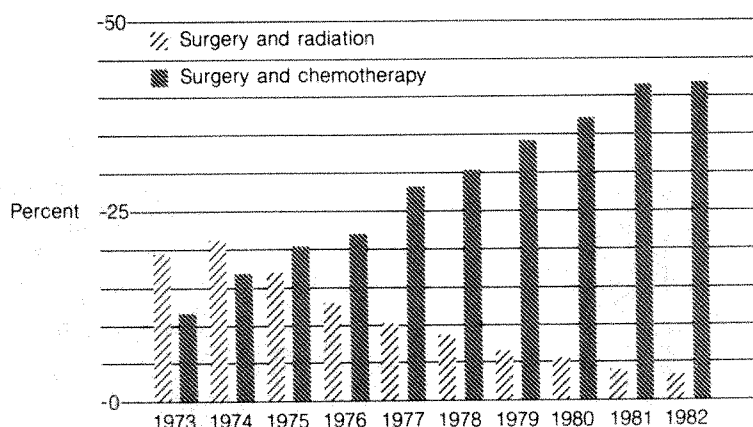
**Stage and age parameters.** Survival after diagnosis of ovarian cancer is a measure of the severity of this malignant neoplasm. Complete 5-year survival experience data are available for 8336 white cancer patients. Table IV presents 5-year relative survival rates for all age groups according to stage. For those with Stage I disease, the average rate is 82%; for patients with Stage II disease, the rate is 60%. However, most of the group have had Stages III and IV disease, for which the rate is only 18%.

Five-year relative survival rates for patients in Stages III and IV range from a high of 41% for the youngest age group to a mere 11% for the patients 65 to 74 years of age, followed by 7% for those 75 years and older. There is a dramatic stepwise decrease in survival rates as age advances. Low stage is associated with more favorable prognosis even for older women in Stages I and II. Patients presenting with advanced disease or with an unknown stage have higher mortality rates. The survival figures for older women in these two categories are significantly ( $p < 0.05$ ) poorer. Thus, although the prognosis of patients with advanced malignant tumors is generally poor for all women, it is even worse as age progresses.

**Stage, age, and treatment.** It was observed earlier that women 65 years or older are more likely to be given therapies as single modalities and that use of combined treatment modalities including surgical procedures decreases as age increases. The survival outcome data of 5612 ovarian cancer patients divided into age groups under 65 years and those 65 or older in Stages III and IV are compared in Fig. 6 to examine the effects with and without surgical treatment. If patients received a surgical procedure as a single interaction or in combination with other therapies, the cases were placed in the surgery category; almost 65% were in this category.

Not only is there a significant difference in survival ( $p < 0.001$ ) between the two age groups, but those under 65 years constitute two thirds of the category receiving surgical treatment. The 5-year relative survival rate for women 65 years or older is almost one half of the rate observed for women under 65.

In comparing the same two age groups according to whether surgical treatment was received, the proportions are reversed. More than 60% of those with Stages III and IV disease receiving no surgical treatment were 65 years or older. In the first year after diagnosis, younger women appear to have better survival outcome than



Source: NCI SEER Program, N = 11,076

**Fig. 5.** Trends in treatment by year of diagnosis: a comparison of surgery with radiation and surgery with chemotherapy, 1973 to 1982.

**Table IV.** Relation of stage to survival rate according to age\*

Stage	Age (yr)	No. of cases†	5-year relative survival rate (%)
I (n = 2074)	<45	565	87
	45-54	505	84
	55-64	502	75
	65-74	275	79
	≥75	227	83
II (n = 247)	<45	36	66
	45-54	64	64
	55-64	73	56
	65-74	50	56
	≥75	24	59
III-IV (n = 5618)	<45	594	41
	45-54	1038	24
	55-64	1592	16
	65-74	1392	11
	≥75	1002	7
Stage unknown (n = 397)	<45	47	56
	45-54	68	33
	55-64	82	30
	65-74	91	13
	≥75	109	16

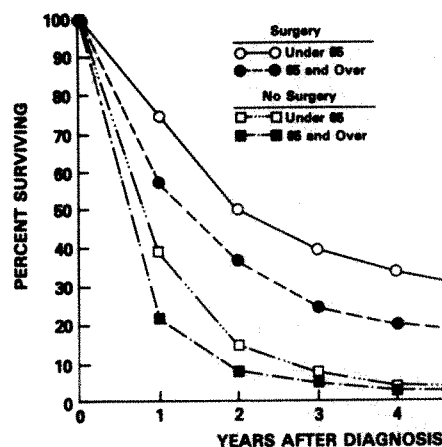
\*Source: National Cancer Institute SEER Program, 1973-1980.

†Total = 8,336.

older women; the difference is significant ( $p < 0.001$ ). With the passage of subsequent years, the differences level off with 3% to 4% surviving in both age groups after 3 years.

#### Comment

SEER Program data aptly demonstrate the relationship of age to the diagnosis and treatment of ovarian cancer. The study points out that older women present with advanced cancers more frequently and are likely to be treated more conservatively than younger ovarian cancer patients.



Source: NCI SEER Program, 1973-80

**Fig. 6.** Relative survival rates by age: Stages III and IV, with and without surgery.

A summary of other findings includes the following: Women 65 years or older are more likely to be in later stages of disease. Of the unstaged cases, over 50% were 65 years or older. Younger women receive more treatment and show better survival rates. Of the women receiving no known therapy, 76% were 65 years of age or older; this compares to 1% who were under age 45. Within advanced disease stages, elderly women fare much worse in terms of survival.

Certainly the data suggest that early detection of these tumors in older population subgroups needs emphasis. The data also imply that older women may not be receiving sufficient staging workups or therapy.

Also shown are the dramatic age-related decreases in survival for comparably staged and treated ovarian cancer patients. Although numerous studies of ovarian malignancy have been performed, rarely do they give heed to age as a significant factor in clinical exper-

ence. With few exceptions (for example, Holmes and Hearne<sup>29</sup>), age contrasts in diagnosis, treatment, and survival have been ignored in the clinical treatment literature. When age considerations are addressed, erroneous extrapolations from past clinical experience from limited studies may occur. Selection bias resulting from placing physiologically younger patients on clinical trials research protocols is common. For example, Malfetano<sup>30</sup> stated in his recent review of the diagnosis and treatment of ovarian cancer that "ninety percent of ovarian neoplasms are found in patients between the ages of 20 and 65." SEER Program data show that 61% were between the ages of 20 and 65 at diagnosis, 38% were 65 years or older, and the remaining 1% were under 20 years.

In *Clinical Oncology for Medical Students and Physicians*, Rubin and Bakemeier,<sup>31</sup> with use of a different age breakdown, report that 60% of ovarian cancers occur between ages of 40 and 60 years, with about 20% occurring under age 40 and 20% over age 60. By contrast, when the SEER Program data were examined with use of the same age breakdown, it was found that 40% of ovarian cancers were diagnosed between ages of 40 and 60 years, with about 10% occurring under age 40 and 50% over age 60, a 30% discrepancy for this older age segment.

The SEER Program data clearly call into question the more prevalent notion persistent in the clinical literature that ovarian cancer is a disease of younger women. Ovarian therapeutic trials report findings that focus on younger women. Also, as pointed out earlier, there is no consistency in reporting age.

This SEER Program study points out treatment differences that occur in persons of different age groups. For whatever reasons, it appears that older persons present with advanced cancers or are treated more conservatively than younger ovarian cancer patients. Intercurrent disease and the pathologic conditions of normal aging that impinge on older persons may account for this finding. Certainly, for the aged women with extremely advanced disease, the toxicity of chemotherapy far outweighs its potential benefit.

The study, which has stratified a large amount of ovarian cancer patient data according to the characteristics of age and stage of disease, suggests two very important age-related issues. Ovarian cancer, known to spread quickly to other organs and to lack early warning signs and symptoms, may be even more insidious in onset and elusive to detect in older women. Progressive age-related deteriorative changes affecting bodily functions may influence the manifestation of ovarian cancer at initial presentation. Additionally, the question arises whether ovarian cancer as a disease phenomenon presents differently because of "the aged host" or possibly

the older person's reactions to and perceptions of the disease symptoms.

SEER Program data raise issues for targeting clinical trials, cancer control, and epidemiologic investigations of ovarian cancer, such as the following: Are older women less attentive to signs and symptoms of disease? Do older women come in later for disease diagnosis because of lack of physician access? Are older women examined less thoroughly (that is, pelvic examinations infrequently given)? Are elderly women treated less aggressively for ovarian cancer?

We believe this descriptive analysis from the SEER Program population-based data set reflects more accurately than do treatment studies, with their limited numbers and age selection biases, the strong association of old age with advanced ovarian cancer. The study emphasizes the extreme importance of detecting localized ovarian cancer, especially in older women. Certainly, improved prognosis for this disease depends on diagnosis in early stages and appropriate treatment. The SEER Program data base provides a useful background from which to judge the generalizable quality of the clinical trials literature on the diagnosis and treatment of ovarian cancer.

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# Microbial colonization of tailed and tailless intrauterine contraceptive devices: Influence of the mode of insertion in the rabbit

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An experimental rabbit model was developed to study the microbial colonization of intrauterine contraceptive devices. Tailed and tailless devices were surgically inserted into the uterus by two different routes: (1) surgically, directly into the uterine horn, thus avoiding contact with the vaginal and cervical microfloras, or (2) via the vagina and cervix. After 1 to 8 weeks the devices were recovered and prepared for scanning electron microscopy. The surfaces of surgically inserted devices remained uncolonized all through the experiment whereas in those inserted via the cervix microorganisms colonized the core surface as early as 2 weeks after insertion. Our data suggest that in our experimental conditions the mode of insertion appears to be the major factor influencing the microbial colonization of intrauterine contraceptive devices and that the presence of a tail does not seem to play a significant role. (AM J OBSTET GYNECOL 1986;154:648-55.)

**Key words:** Intrauterine contraceptive devices, colonization, microorganisms

Women who use intrauterine contraceptive devices (IUCDs) are at an increased risk of developing genital tract infections.<sup>1,3</sup> The mechanism by which those infections are related to IUCDs is not well understood. The projection of part of the device through the cervical canal is thought by many to allow easy access of vaginal microorganisms to the upper genital tract.<sup>4,5</sup> It is also possible that bacteria could be introduced into the uterus at the time of installation of the device.<sup>3</sup> In the present study we report on an experimental model designed to establish whether the tail or the mode of insertion is the major pathway for the entry of microorganisms into the uterus. The rabbit was chosen because other aspects of IUCD implantation have been studied in this animal.<sup>6-9</sup>

### Material and methods

**Animals.** Forty mature female New Zealand rabbits, 2.5 to 3 kg (4 to 5 months old), were purchased from

Stoney Creek, Chase, British Columbia, Canada, and the Kleefeld Rabbitry, Niverville, Manitoba, Canada.

**IUCD.** The IUCD used in the present study was the Gyne-T 380 (Ortho Pharmaceutical Canada Ltd., Don Mills, Ontario, Canada). The vertical arms of this T-shaped IUCD were shortened in order to fit the uterus of the rabbit, but the T shape had to be maintained to avoid expulsion.

**Insertion of the IUCD.** To compare tailed and tailless IUCDs as well as aseptic insertion and normal insertion via the cervix, the animals were divided into four equal groups. In group 1, a tailless IUCD was surgically inserted into one uterine horn. In group 2, a tailless IUCD was inserted through the cervix into one uterine horn. In group 3, a tailed IUCD was surgically inserted into one uterine horn; the trailing end of the tail extended into the vagina. In group 4, a tailed IUCD was inserted through the cervix into one uterine horn. In this group the tail passed through the cervix and extended into the vagina, which imitates the normal situation in the human.

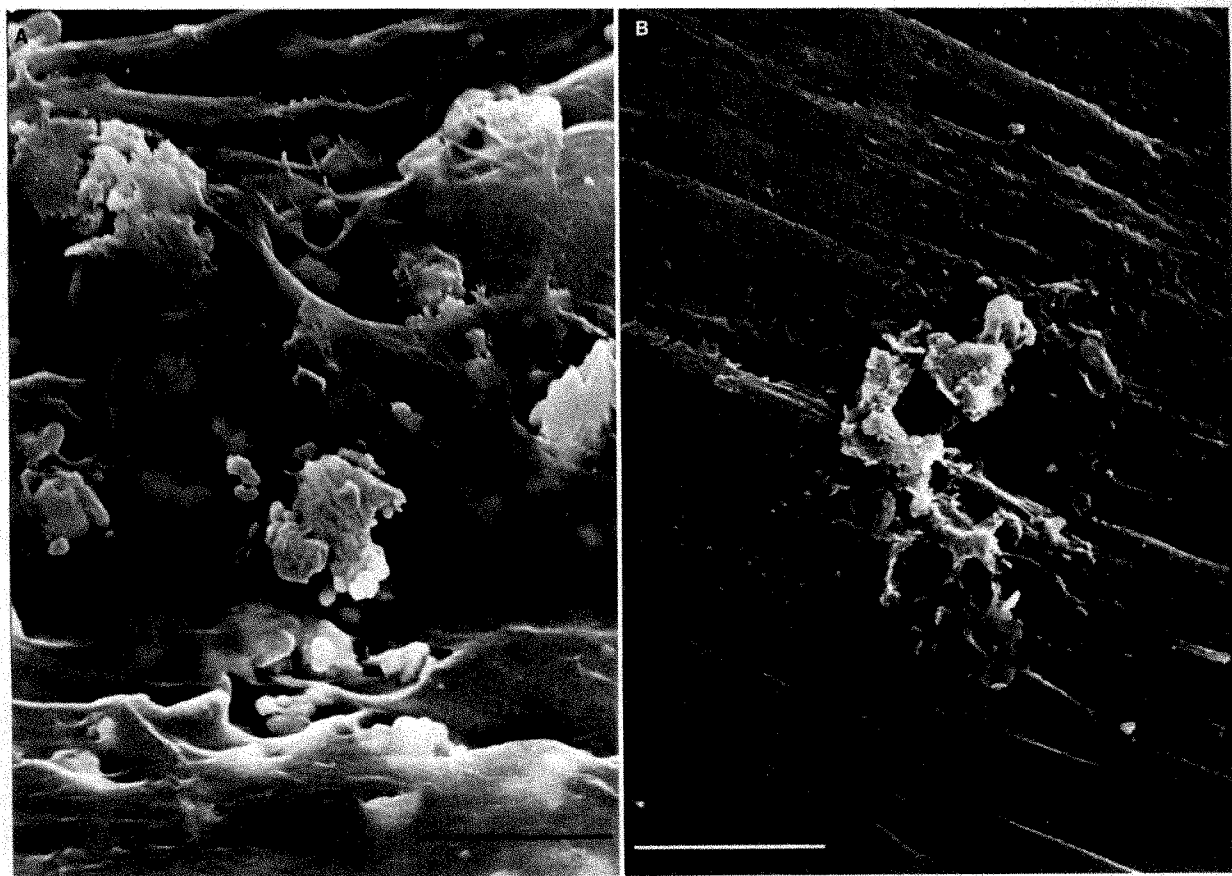
Sterile surgical technique was used to insert the IUCDs with the animals under halothane anaesthesia. The uterine horns were exposed through a midventral incision, and the IUCD was introduced into the uterus through a stab incision in the wall of the lower half of the left uterine horn. This approach avoided contamination of the IUCDs by cervical and vaginal microflora

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**Fig. 1.** Scanning electron micrographs of an unused control IUCD. (A) Note the material protruding at the surface of the core portion of the device. (B) Note the material protruding and the longitudinal cavities at the surface of the tail of the device. Bars represent 5  $\mu$ m.

and was used for the animals of groups 1 and 3. For the animals of groups 2 and 4, IUCDs were introduced through a stab incision in the wall of the vagina and were then pushed through the cervix into the lower half of the left uterine horn. The site of insertion was closed with 5-0 chromic catgut, with a Cushing and Lembert pattern. After 1, 2, 4, 6, and 8 weeks the IUCDs were recovered and prepared for scanning electron microscopy.

**Scanning electron microscopy.** IUCDs were immediately fixed in a solution consisting of 5% glutaraldehyde in cacodylate buffer (0.1 mol/L, pH 7.0) for 2 hours at 20° C. They were washed five times in buffer, dehydrated in ethanol and Freon 113 before critical point drying<sup>10</sup> and "sputter coating" with gold and palladium, and then examined by means of a Hitachi S450 (Hitachi, Rexdale, Ontario, Canada) scanning electron microscope.

## Results

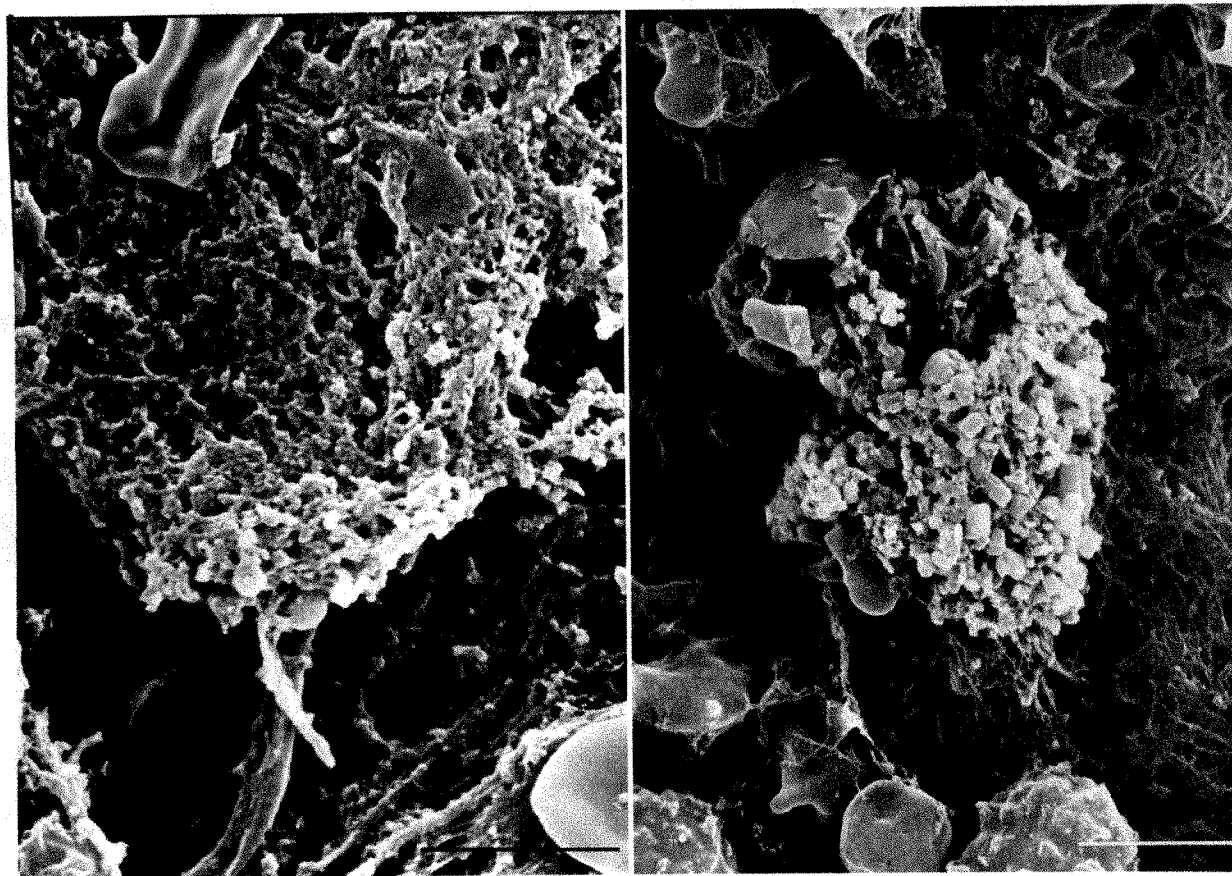
The experimental model used was designed to determine whether the mode of insertion and/or the presence of a tail were involved in microbial colonization of IUCDs. The degree of microbial coloniza-

tion was evaluated by means of scanning electron microscopy.

Unused control IUCDs revealed a rather smooth surface with some protruding material in the case of the core portion (Fig. 1, A) and a surface exhibiting longitudinal cavities as well as protruding material in the case of the tail portion (Fig. 1, B).

The surface areas of the IUCDs showed amounts of cellular and amorphous material that increased with the time the device had been in utero. The core surface of aseptically inserted IUCDs, where contact with vaginal and cervical microfloras was avoided, remained uncolonized throughout the experiment. In contrast, IUCDs contaminated during insertion via the vagina and cervix showed microorganisms colonizing the surface as soon as 2 weeks after insertion.

The core portions of tailed and tailless devices that had been aseptically inserted and had been in situ for 8 weeks are shown in Fig. 2. The heavy biofilm, seen here at a high magnification, consists of amorphous and cellular material, and no bacterial cells were observed. Tailed IUCDs that had been inserted through the vagina and cervix and had been in situ for 4 (Fig. 3, A) and 8 (Fig. 3, C) weeks were entirely covered by a thick



**Fig. 2.** Scanning electron micrographs of aseptically inserted IUCDs that have been in utero for 8 weeks. A heavy biofilm consisting of cellular and amorphous material is entirely covering the core surface of a tailed device (A) and of a tailless device (B). Note the absence of bacterial cells on both preparations. Bars represent 5  $\mu$ m.

biofilm composed of amorphous and cellular material within which bacterial cells were clearly recognizable. Tailless IUCDs that had been inserted through the vagina and cervix and had been in situ for 4 (Fig. 3, B) and 8 (Fig. 3, D) weeks were also entirely covered with a similar bacterial biofilm; no significant differences were noted between the number of bacteria colonizing tailed and tailless IUCDs that had been inserted via the cervical route. The biofilm covering the plastic portion of an IUCD was always observed to be similar (based on the biofilm's appearance and extent) to the one covering its copper portion.

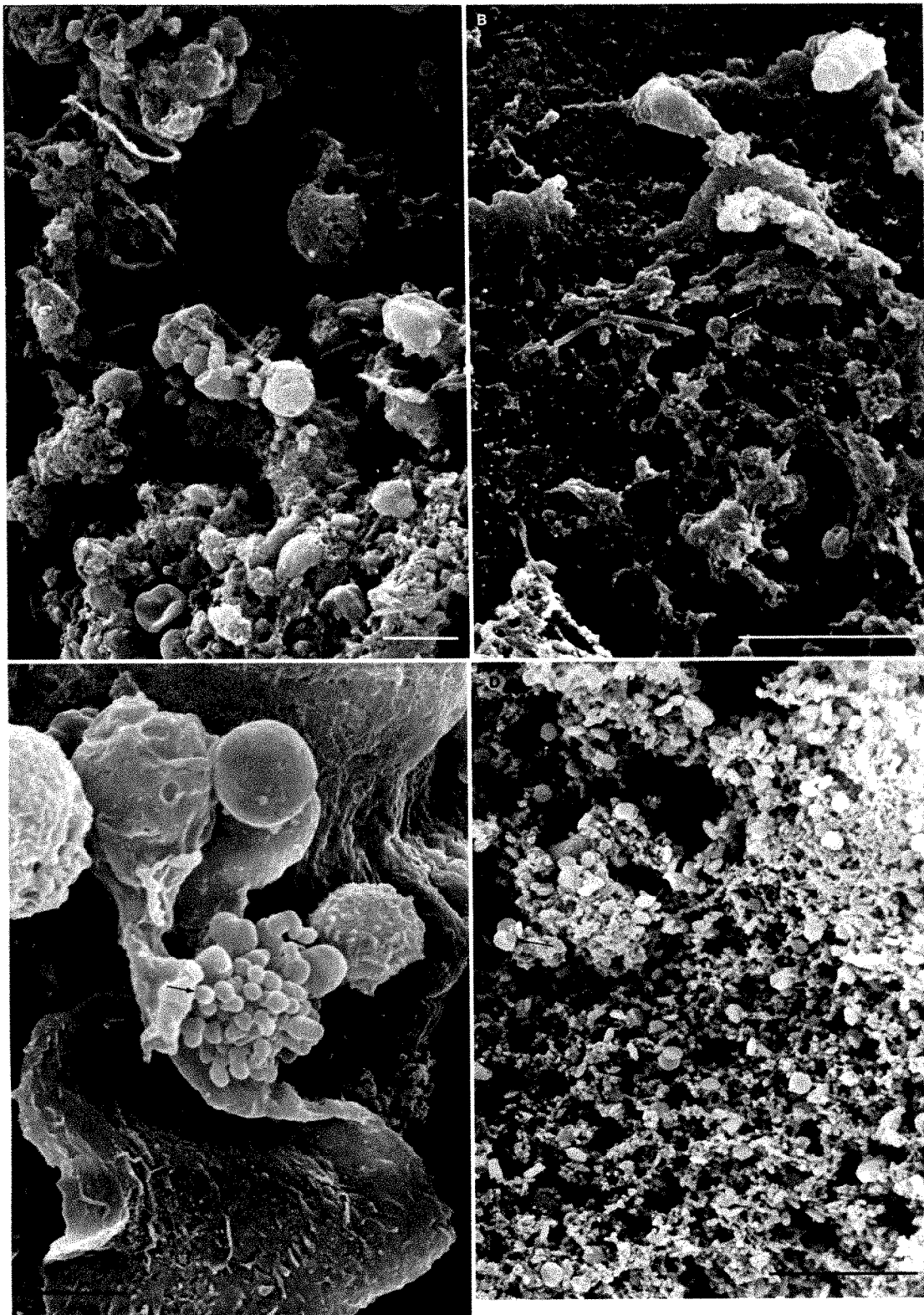
The intravaginal portion of the tailed IUCDs demonstrated a moderate amount of biofilm material overlying the smooth plastic surface (Fig. 4, A); and bacterial cells were often seen associated with this material (Fig. 4, B). In contrast, the portion of the tail close to the cervical canal was so heavily encrusted that the underlying plastic surface was almost invisible and many bacterial cells were observed (Fig. 4, C).

Two of the rabbits with conventionally inserted IUCDs (one in group 2 and one in group 4) developed

an infection of the uterus within 2 weeks after insertion of the device, causing the death of these two animals. Cultures were performed on the fluid and pus contained in the uterine horns. *Staphylococcus epidermidis* was recovered in huge numbers from one rabbit and *Alcaligenes* sp. in the other one; these two microorganisms are part of the normal vaginal microflora of rabbits (Jacques M, et al. unpublished data). Examination with scanning electron microscopy of the devices recovered from these rabbits showed very thick bacterial biofilms (Fig. 5, A) that were almost completely covered by leukocytes (Fig. 5, B). These biofilms were thicker and heavier than the ones observed on devices inserted according to the same experimental conditions but recovered from a healthy rabbit (Fig. 5, C).

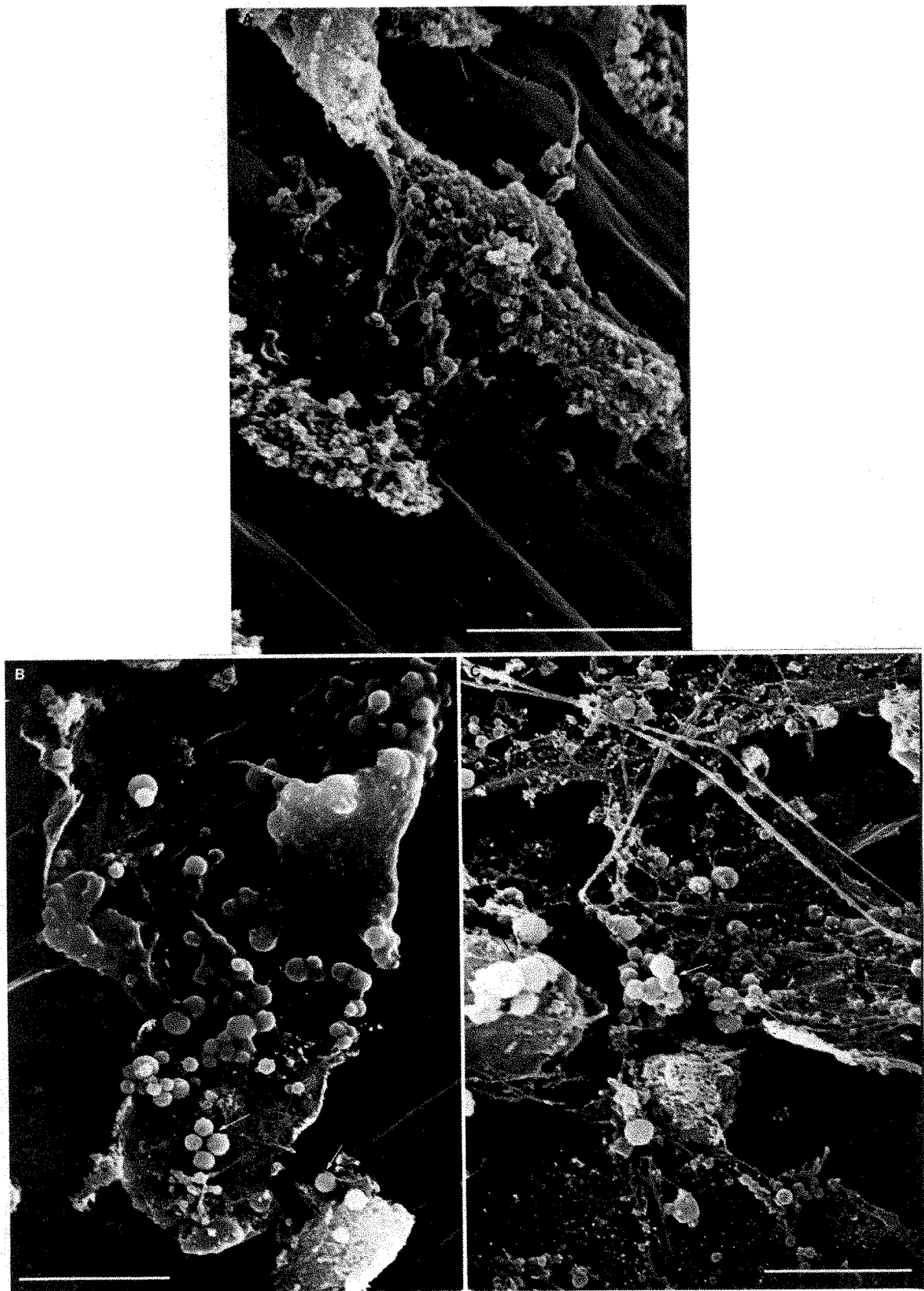
#### Comment

It is generally assumed that in humans the endometrial cavity is usually sterile.<sup>11</sup> Previous studies have focused on the way bacteria are introduced into the uterine cavity of IUCD users. Mishell et al.<sup>11</sup> obtained cultures from hysterectomy specimens and found bac-

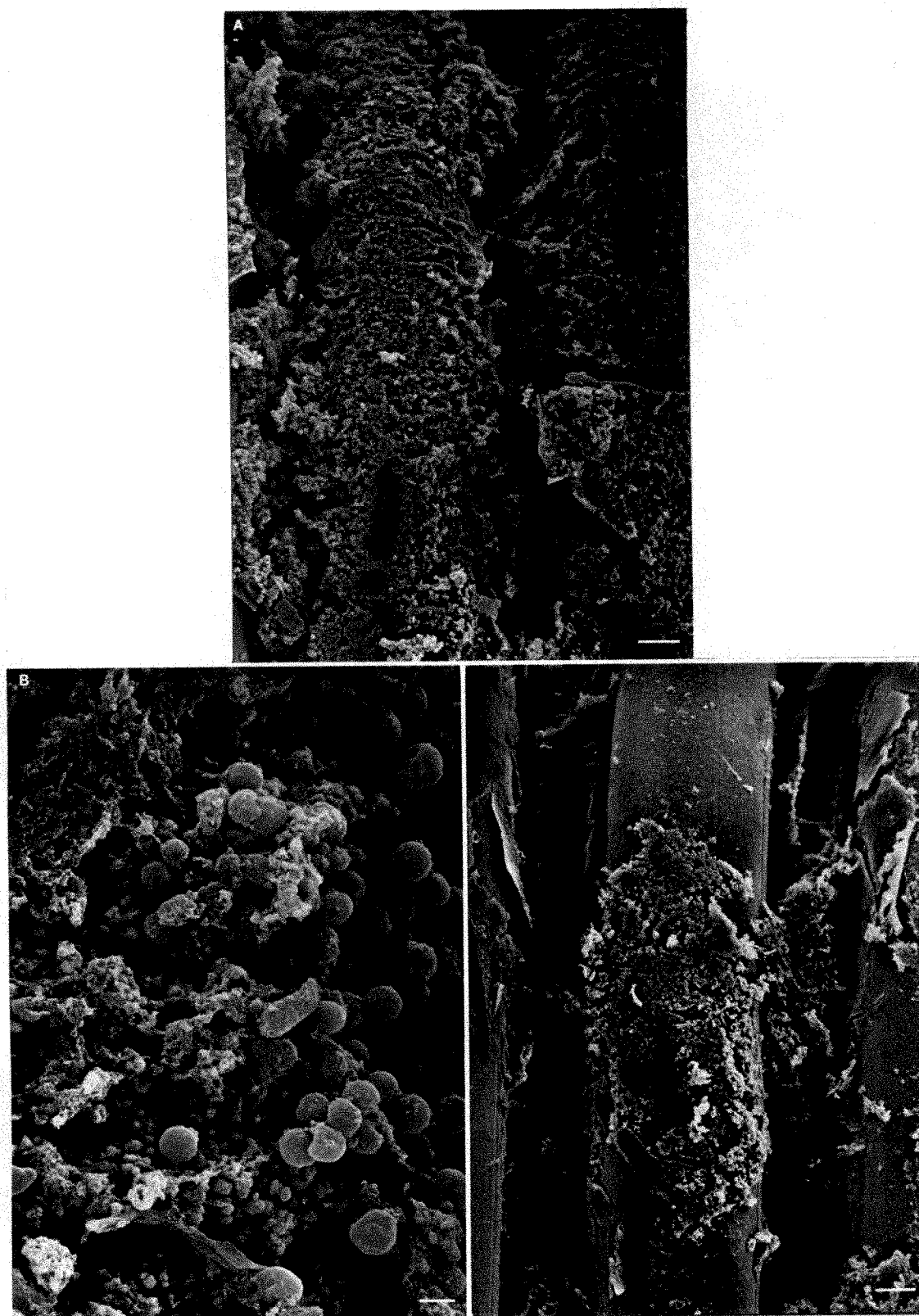


**Fig. 3.** *A and B*, Scanning electron micrographs of unseptically inserted IUCDs that have been in utero for 4 weeks. The core surface of a tailed device (*A*) and of a tailless device (*B*) is completely covered with a heavy biofilm consisting of cellular and amorphous material. Some bacterial cells are recognizable (*arrows*). *C and D*, Scanning electron micrographs of unseptically inserted IUCDs that have been in utero for 8 weeks. The core surface of a tailed device (*C*) and of a tailless device (*D*) is completely covered with a heavy biofilm consisting of cellular and amorphous material and in which bacterial cells are seen (*arrows*). Bars represent 5  $\mu\text{m}$ .





**Fig. 4.** Scanning electron micrographs of different portions of the tail of an IUCD that has been in utero for 4 weeks. (A) Intravaginal portion of the tail, which demonstrated a moderate amount of material; no bacterial cells are seen. (B) Intravaginal portion of the tail that also demonstrated a moderate amount of material, but this time some bacterial cells are seen (*arrows*). (C) The portion of the tail close to the cervical canal was almost completely covered with material, and a large number of bacterial cells was seen (*arrows*). Bars represent 5  $\mu$ m.



**Fig. 5.** Scanning electron micrographs of the copper coil of unaseptically inserted tailed IUCDs that have been in utero for 1 week. (A) A very large biofilm is completely covering the surface of this device removed from a rabbit that died and from which *Staphylococcus epidermidis* was cultured. (B) At higher magnification, large numbers of leukocytes are seen. (C) The surface of this device, which was removed from a healthy rabbit, is only partly covered by a biofilm. Bars represent 5  $\mu\text{m}$ .

teria in all the uterine cavities examined within 24 hours of IUCD insertion. They concluded that IUCD insertion introduced bacteria that disappeared within a few weeks from the cavity. The tail also represents a potential route along which microorganisms might ascend from the vagina to the uterine cavity. Sparks et al.<sup>5</sup> examined the effect of various types of tailed and tailless IUCDs on the bacteriologic status of the uterus; all five uteri with a tailless IUCD were sterile but 15 of 17 with a tailed device contained bacteria. The bacteria found do not represent the survival of organisms introduced at insertion as they are absent from the uteri with a tailless IUCD; clearly the tail was implicated in the introduction of bacteria into the uterine cavity. Skangalis et al.<sup>12</sup> found that insertion of IUCDs could promote entry of bacteria in the uterine cavity of baboons, but the retrieval tail was the principal factor implicated. Purrier et al.<sup>4</sup> reported an *in vitro* model designed to establish whether bacteria ascend in the mucus that coats the IUCD tail after insertion of the device. They found that potentially pathogenic bacteria colonized the mucus coating the tails of the IUCDs in 55% of tests. They suggested that the IUCD tail may be responsible for the passage of vaginal bacteria into the uterus.

Much of the present uncertainty surrounding the introduction of bacteria into the uterine cavity is due to the fact that most of the relevant studies have been done in humans and so are necessarily limited in experimental flexibility. Our experimental model in rabbits was designed to establish whether the mode of IUCD insertion or the presence of a tail was the major factor influencing microbial colonization of the devices.

We used scanning electron microscopy to examine the surfaces of IUCDs that have been implanted in rabbits for 1 to 8 weeks and gave particular attention to microbial colonization of these devices. Previously published studies of the surface areas of various IUCDs used for varying lengths of time have demonstrated the presence of adherent material.<sup>14, 15</sup> Major areas of interest to date have included the analysis of the chemical nature of the surface encrustation and the elucidation of the cellular components of the proteinaceous material found on some IUCDs shortly after their removal. All devices examined exhibited varying amounts of surface calcium deposition.<sup>14, 15</sup> The cellular material identified on the surface areas of inert IUCDs consisted mainly of macrophages, with some polymorphonuclear leukocytes, erythrocytes, a few platelets, and fibrin fibers.<sup>15, 16</sup> Marrie and Costerton<sup>17</sup> observed different morphologic types of bacteria adherent to the devices they examined.

In the present study, examination of a series of tailed and tailless IUCDs that had been implanted for several weeks revealed that biofilm formation occurred pro-

gressively on aseptically and conventionally inserted IUCDs and that microbial colonization occurred progressively on the latter IUCDs only. Our data suggest that the mode of insertion is the primary and also the major factor involved in microbial colonization of IUCDs and that the presence of a tail, forming a bridge between the vagina and the uterine cavity, may permit propagation of some bacteria on the uterine portion of a device that was already contaminated during insertion.

Our observation of different degrees of biofilm formation and microbial colonization of different sections of the tail can be explained by the fact that wide variations exist in the microenvironments with which an IUCD is in contact.<sup>13</sup> The leading end of the tail is in the uterine cavity while the middle portion lies in the endocervical canal, and the trailing portion hangs within the vagina. The variation in specific environments includes not only biochemical differences but also differences in the microflora.

Tatum<sup>18</sup> published an in-depth consideration of the many possible problems associated with IUCDs. He stated that IUCDs cannot usually be inserted without some introduction of bacteria from the vagina and external cervical os and that monofilament tails did not seem to provide "ladders" for the ascend of bacteria into the uterine cavity after insertion but that both fluids and bacteria could readily ascend multifilament tails by capillary action. Our results fully support Tatum's first two conclusions and do not apply to the third because multifilament tails were not included in our study.

In our experimental conditions, the mode of insertion appears to play the predominant role in microbial colonization of IUCDs. Avoiding mechanical transfer of bacteria to the usually sterile uterine cavity as well as developing new materials less prone to microbial colonization will hopefully lead to a reduction in pelvic infections among IUCD users.

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## Effects of reduced uterine blood flow on electrocortical activity, breathing, and skeletal muscle activity in fetal sheep

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Experiments were conducted in 11 unanesthetized fetal sheep during the last third of gestation to examine the effects of prolonged, reversible reduction in uterine blood flow on fetal electrocortical activity, breathing movements, and skeletal muscle activity. With an adjustable clamp placed around the maternal common internal iliac artery, uterine blood flow was restricted for 2 hours to produce a reduction in fetal arterial oxygen saturation from  $56.1\% \pm 1.9\%$  to  $28.8\% \pm 0.7\%$ . When blood flow was reduced, there was a decrease in the percentage of time that fetuses spent in low-voltage electrocortical activity, from  $57.5\% \pm 3.0\%$  to  $37.8\% \pm 3.5\%$ , and a decrease in the incidence of both breathing movements and integrated skeletal muscle activity. Younger fetuses (110 to 121 days' gestation) demonstrated a lesser degree of reduction in breathing movements when compared with older fetuses (125 to 140 days) whereas the effects of hypoxemia on electrocortical activity became less apparent with advancing gestational age. (*AM J OBSTET GYNECOL* 1986;154:655-62.)

**Key words:** Sleep states, fetal activity, hypoxia, uterine blood flow

Maternal vascular disease, which may lead to a restriction in blood flow to the gravid uterus, is often associated with intrauterine growth retardation in the human<sup>1</sup> and therefore with an increase in perinatal morbidity and mortality. An understanding of fetal

physiologic responses to reduced uterine blood flow in experimental animals may help in the interpretation of changes in biophysical parameters that are observed in clinical conditions. Two commonly used indicators of fetal well-being in the human are the incidence of fetal breathing movements and the incidence of fetal body movements.<sup>2,3</sup> We have investigated the effects of controlled reductions in uterine blood flow on fetal breathing movements and skeletal muscle activity in pregnant sheep. Since these physiologic functions are closely linked to fetal sleep states,<sup>4</sup> we have also documented the changes in electrocortical activity and eye movements when uterine blood flow is reduced.

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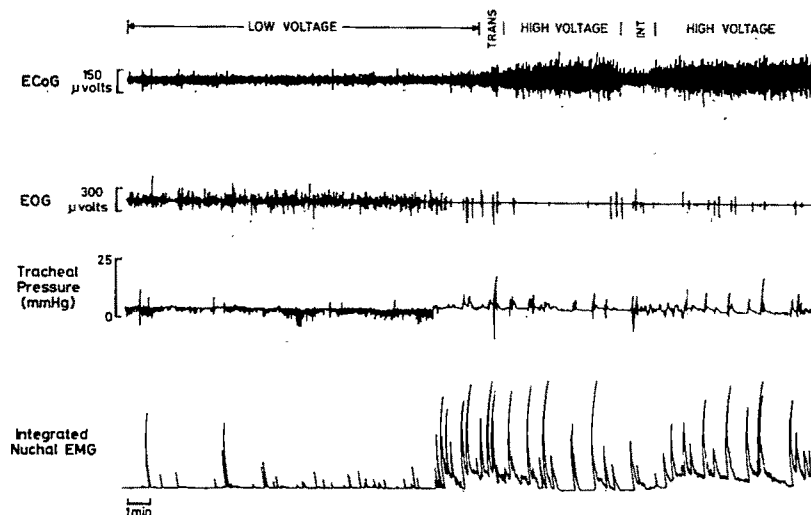


Fig. 1. Electrocorticogram (ECoG), electrooculogram (EOG), tracheal pressure (with amniotic pressure electronically subtracted), and integrated nuchal electromyogram (EMG) in a sheep fetus at 137 days. The classifications of electrocortical activity into low-voltage, high-voltage, transitional, and intermediate states are indicated at the top.

Previous studies on fetal behavioral responses to reduced oxygen delivery in sheep have been conducted during maternal hypoxemia induced by having the ewe inspire a low-oxygen-gas mixture.<sup>5-8</sup> These studies have shown that fetal hypoxemia leads to a decrease in the incidence of breathing and body movements although there are conflicting reports as to the effects of hypoxemia on fetal sleep states.<sup>5,7,8</sup> In addition to these behavioral changes in the fetus, maternal hypoxemia is associated with an increase in plasma cortisol concentrations and metabolites in the ewe.<sup>9,10</sup> These changes may have an effect on either fetal or placental metabolism and therefore indirectly on fetal behavior. Maternal hypoxemia is also a condition that rarely occurs in the human. By using an adjustable clamp placed around the major artery supplying blood to the pregnant uterus,<sup>11</sup> we have been able to produce prolonged reversible hypoxemia in fetal sheep without the endocrine changes associated with maternal hypoxemia.<sup>12</sup>

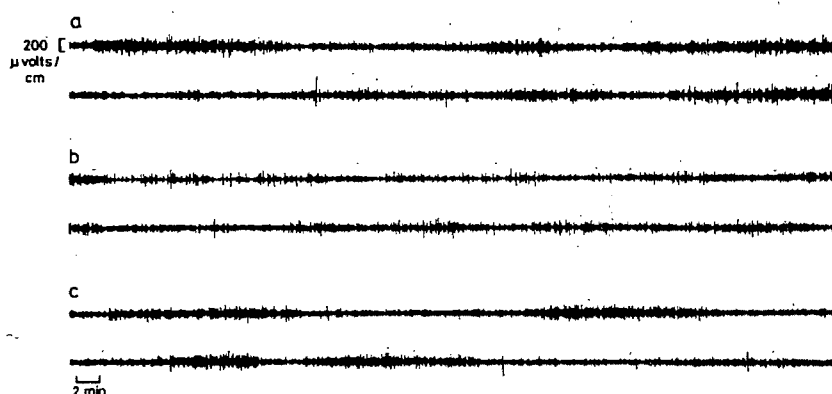
Since sleep states and breathing and body movements are known to be episodic and have a periodicity of 20 to 40 minutes in fetal sheep,<sup>4</sup> we chose 2 hours as the minimum duration of hypoxemia required to reveal changes in these physiologic functions. In addition, we hypothesized that if the sheep fetus is able to adapt behaviorally to a reduction in oxygen delivery, this may become evident during the second hour of reduced uterine blood flow.

#### Material and methods

**Animal preparation.** Operation was performed on 11 pregnant sheep of known mating dates between 105 and 125 days' gestational age (mean 113 days). With

the animals under halothane anesthesia, a midline incision was made in the abdomen of the ewe and a Teflon vascular clamp was placed around the maternal common internal iliac artery.<sup>11,12</sup> An incision was then made in the uterus, and polyvinyl catheters (SV65, Dural Plastics) were placed either in the fetal carotid artery and jugular vein ( $n = 7$ ) or in the descending aorta and inferior vena cava ( $n = 4$ ). Fine stainless steel wires (Cat. No. AS632, Cooner Sales Co.) were placed over the dura of the parietal cortex approximately 10 mm on either side of the midline for recording the electrocorticogram and in the inner and outer canthi of one eye for recording the electrooculogram. Pairs of electrodes were also sutured into the nuchal ( $n = 11$ ), biceps and triceps ( $n = 8$ ), and semitendinosus and vastus medialis muscles ( $n = 4$ ) for electromyographic recordings. Polyvinyl catheters (SV115, Dural Plastics) were placed in the fetal trachea, amniotic sac, and maternal carotid artery and jugular vein in all animals. At the end of operation, the fetal catheters and wires, as well as the control cable for the vascular clamp, were exteriorized through the flank of the ewe and antibiotics (benzylpenicillin, 400,000 units, and streptomycin, 500 mg) were injected into the amniotic sac.

The ewes were housed in individual cages with free access to food and water and were allowed 5 days to recover from operation before any experiments were performed. Nine fetuses survived until term (140 to 145 days' gestation) and two ewes were electively killed prior to 140 days. One of these fetuses weighed 2.5 kg at 135 days and the other weighed 3.9 kg at 137 days with marked ascites, probably as a consequence of the catheters having become tightly wrapped around the



**Fig. 2.** Electrocardiogram activity in a sheep fetus of 121 days' gestation in an early stage of differentiation. Each pair of lines represents 120 minutes of recording before (a), during (b), and after (c) reduction in uterine blood flow. In b there is a reduction in the voltage amplitude and a loss of high-voltage electrocardiogram activity.

**Table I.** Fetal arterial blood gases and percentage of  $\text{SaO}_2$  before (–15 minutes), during (+15, +60, +120 minutes), and after (p30 minutes) the 2-hour period of reduced uterine blood flow. Results are mean values  $\pm$  SEM (n = 25, 11 fetuses)

	Time (min)				
	t = –15	t = +15	t = +60	t = +120	t = p30
$\text{SaO}_2$ (%)	56.1 $\pm$ 1.9	27.4 $\pm$ 1.2*	29.6 $\pm$ 1.2*	29.3 $\pm$ 1.5*	48.3 $\pm$ 2.1*
$\text{PO}_2$ (mm Hg)	22.3 $\pm$ 0.5	14.9 $\pm$ 0.6*	16.4 $\pm$ 0.6*	16.5 $\pm$ 0.6*	21.3 $\pm$ 0.7†
$\text{PCO}_2$ (mm Hg)	46.3 $\pm$ 1.2	51.2 $\pm$ 1.4‡	50.2 $\pm$ 1.1‡	48.2 $\pm$ 1.2	45.7 $\pm$ 1.3
pH	7.35 $\pm$ 0.01	7.27 $\pm$ 0.01*	7.26 $\pm$ 0.01*	7.26 $\pm$ 0.01*	7.31 $\pm$ 0.01†

\*p < 0.001 (paired t test for comparison with t = –15 minutes).

†p < 0.05 (paired t test for comparison with t = –15 minutes).

‡p < 0.01 (paired t test for comparison with t = –15 minutes).

neck of the fetus. The mean weight of the nine fetuses at term was  $4.0 \pm 0.5$  kg.

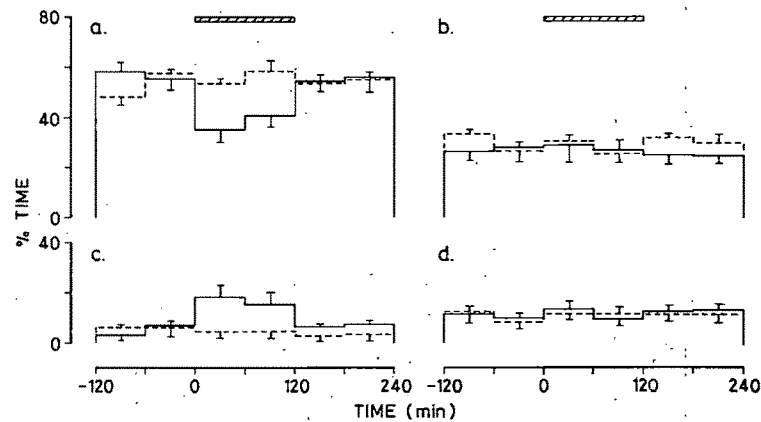
**Experimental protocol.** All studies began at 10:00 AM and consisted of an initial 2-hour data collection period, a 2-hour period during which uterine blood flow was reduced sufficient to produce a fetal arterial oxygen saturation ( $\text{SaO}_2$ ) of approximately 30%, and a 2-hour recovery period.

Fetal and maternal arterial blood samples were obtained at 15 minutes before (–15) and at 15, 60, and 120 minutes during the reduction in uterine blood flow and at 30 minutes after the release of the vascular clamp. On separate days, control recordings were made, during which blood flow was not altered in order to minimize the risk of attributing the effects of experimental maneuvers to diurnal changes in electrocardiogram activity and fetal breathing movements. Each animal underwent an average of four (range two to eight) experiments in which uterine blood flow was reduced and at least 48 hours of recovery was allowed before further recordings were performed in the same fetus.

**Data analysis.** The electrocardiogram was recorded

with the use of AC electroencephalogram preamplifier (Model 7P5B, Grass Instruments) on a polygraph at a paper speed of 10 mm/min. For each period of recording, a maximum voltage threshold for a low-voltage, fast-wave electrocardiogram was determined during the initial 2 hours. This threshold, which ranged from 45 to 150  $\mu\text{V}$ , varied between fetuses and with gestational age. A minimum threshold for a high-voltage, slow-wave electrocardiogram was also determined and ranged from 90 to 330  $\mu\text{V}$ . Two additional classifications of electrocardiogram activity were defined when the voltage was between the threshold for recognition of low- and high-voltage activity. These have been called transitional electrocardiograms if between an episode of low- and high-voltage electrocardiogram activity and intermediate electrocardiograms if within an episode of either a low-voltage or a high-voltage electrocardiogram activity (Fig. 1). In order for a change of classification to have taken place, the voltage change must have been present for at least 30 seconds.

Tracheal pressure (with amniotic pressure electronically subtracted) was displayed continuously on the polygraph. Episodes of fetal breathing movements



**Fig. 3.** Histograms of the percentages of time spent in low-voltage electrocortical activity (a), high-voltage electrocortical activity (b), intermediate electrocortical activity (c), and transitional electrocortical activity (d) before, during, and after a 2-hour episode of reduced uterine blood flow. Means  $\pm$  SEM are plotted for 14 experiments in six fetuses during which uterine blood flow was reduced (—) and for 13 control recordings in seven fetuses where uterine blood flow was not altered(---). The hatched bar indicates the time during which uterine blood flow was reduced.

**Table II.** Frequency and mean duration of episodes of low-voltage, high-voltage, intermediate, and transitional electrocortical activity before ( $-120 \rightarrow 0$ ), during ( $0 \rightarrow 120$  minutes), and after ( $120 \rightarrow 240$  minutes) reduced uterine blood flow

Electrocortical activity	Time period		
	$-120 \text{ min} \rightarrow 0$	$0 \rightarrow 120 \text{ min}$	$120 \rightarrow 240 \text{ min}$
Low-voltage			
Episodes/hr	$2.9 \pm 0.2$	$3.1 \pm 0.3$	$2.6 \pm 0.2$
Mean duration (min)	$13.1 \pm 1.4$	$8.2 \pm 1.0^*$	$14.9 \pm 2.1$
High-voltage			
Episodes/hr	$2.3 \pm 0.2$	$2.0 \pm 0.3$	$2.5 \pm 0.4$
Mean duration (min)	$7.3 \pm 0.7$	$8.2 \pm 0.8$	$7.3 \pm 0.8$
Intermediate			
Episodes/hr	$0.8 \pm 0.2$	$1.5 \pm 0.2$	$1.4 \pm 0.4$
Mean duration (min)	$3.7 \pm 0.9$	$4.9 \pm 1.4$	$2.9 \pm 0.6$
Transitional			
Episodes/hr	$2.8 \pm 0.2$	$2.4 \pm 0.4$	$2.8 \pm 0.3$
Mean duration (min)	$1.9 \pm 0.2$	$2.6 \pm 0.4$	$2.2 \pm 0.6$

\* $p < 0.01$  (paired  $t$  test for comparison to  $-120 \text{ minutes} \rightarrow 0$ ).

were defined as repeated negative fluctuations in tracheal pressure of  $>2$  mm Hg lasting for 30 seconds or longer. Deep inspiratory efforts were defined as isolated negative fluctuations in tracheal pressure of  $>10$  mm Hg.

The electromyographic activity in nuchal and limb muscles was summed over 30-second intervals with the use of an integrator circuit. Samples of the integrated signal were then transferred to a large time-shared computer (VAX 11/780, DEC Corp.) for storage and later analysis. Results of electromyographic activity are presented as the percentage of the control value, which was the mean of all 30-second samples ( $n = 240$ ) during the initial 2-hour recording period for both control and partial occlusion experiments.

Arterial  $\text{PO}_2$ ,  $\text{PCO}_2$ , and pH were measured with ei-

ther a Corning 165/2 or a Radiometer ABL30 blood gas analyzer at  $37^\circ \text{C}$  and then corrected for a fetal temperature of  $39^\circ \text{C}$ . Arterial  $\text{SaO}_2$  was measured with a Radiometer OSM2 Oximeter. All results are presented as means  $\pm$  SEM and statistical significance was determined by means of a paired or nonpaired  $t$  test as appropriate.

## Results

**Blood gases and  $\text{SaO}_2$  percentages.** Twenty-five experiments were performed in the 11 fetuses between 111 and 142 days' gestation. Reduced uterine blood flow caused a decrease in  $\text{SaO}_2$ ,  $\text{PO}_2$ , and pH and a small increase in  $\text{PCO}_2$  (Table I).  $\text{SaO}_2$ ,  $\text{PO}_2$ , and pH remained lower than control values ( $-15$  minutes) 30 minutes after the release of the clamp. During 28 con-

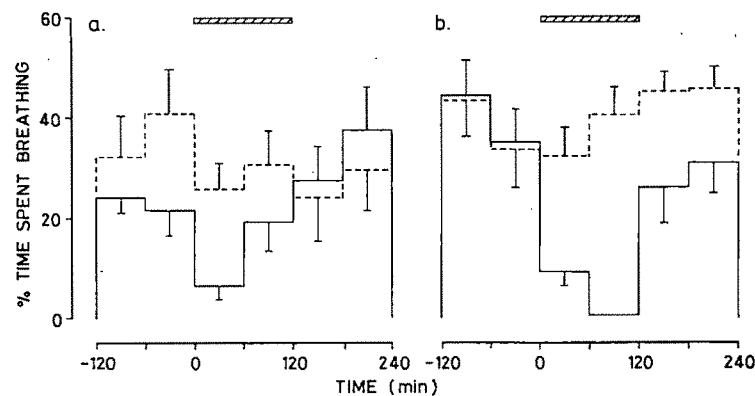


Fig. 4. Histograms of the percentage of time spent making breathing movements for fetuses of 110 to 121 days' gestation (a) and 125 to 141 days' gestation (b). There were six experiments in four fetuses for each of the groups when uterine blood flow was reduced (—) as well as for control recordings (---). The hatched bar indicates the time during which uterine blood flow was reduced.

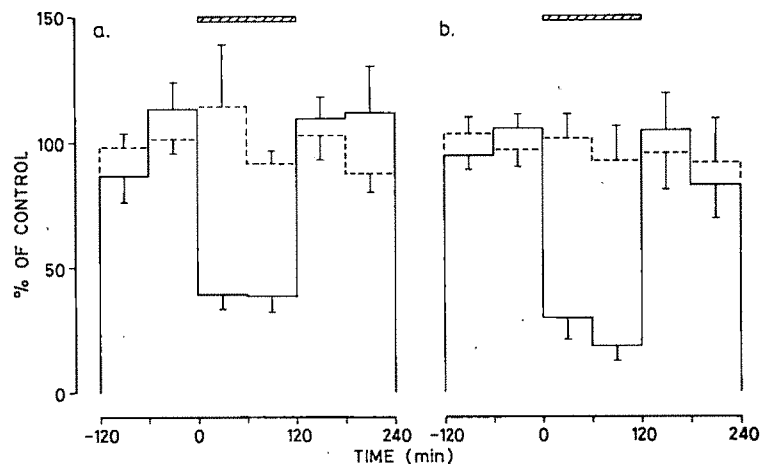


Fig. 5. Histograms of the integrated electromyogram of (a) nuchal ( $n = 9$ , six fetuses, 117 to 142 days' gestation) and (b) limb ( $n = 7$ , four fetuses, 120 to 142 days' gestation) muscles expressed as a percentage of control values during reductions in uterine blood flow (—) and during control recordings (---). The mean value of each 30-second sample during the first 2 hours of recording was taken as 100%. The hatched bar indicates the time during which uterine blood flow was reduced.

control recordings, fetal arterial  $\text{SaO}_2$ ,  $\text{PO}_2$ ,  $\text{PCO}_2$ , and pH were  $56.2\% \pm 1.7\%$ ,  $22.2 \pm 0.5$  mm Hg,  $46.1 \pm 1.3$  mm Hg, and  $7.35 \pm 0.01$ , respectively. No significant change occurred in maternal arterial  $\text{SaO}_2$ , blood gases, or pH when uterine blood flow was reduced.

**Electrocortical activity.** Satisfactory recordings of electrocortical activity were obtained in 20 experiments in eight fetuses. Six recordings were obtained during the early stages of differentiation into low- and high-voltage electrocortical activity (gestational age 111 to 121 days). In three of these experiments, there was a decrease in the voltage of the electrocortical activity and occasionally the electrocortical activity became isoelectric for brief periods (30 to 60 seconds) when uterine blood flow was reduced. Fig. 2 is an example of a partially differentiated electrocorticogram in a fetus at 121

days' gestation in which there was a small reduction in the voltage and a loss of high-voltage periods. In the remaining three experiments, reducing uterine blood flow had no effect on the electrocorticogram.

Fourteen experiments were conducted in six fetuses at an age when the electrocortical activity had clearly differentiated (gestational age 123 to 141 days). There was a decrease in the percentage of time that the fetuses spent in low-voltage electrocortical activity, from  $57.5 \pm 3.0$  during the 2-hour control period to  $35.0 \pm 4.7$  ( $p < 0.01$ ) and  $40.5 \pm 3.8$  ( $p < 0.02$ ) during the first and second hours, respectively, of reduced uterine blood flow (Fig. 3). After the release of the clamp, the percentage of time spent in low-voltage electrocortical activity returned to control levels. There was a decrease in the mean duration of low-voltage epi-



sodes, from  $13.7 \pm 1.3$  to  $8.3 \pm 0.9$  minutes, when uterine blood flow was reduced ( $p < 0.01$ ) although the mean number was not affected (Table II).

The percentage of time that fetuses spent in high-voltage electrocortical activity was not always affected in the same way by a reduction in uterine blood flow. In seven experiments, there was a decrease in the incidence of high-voltage electrocortical activity, from  $28.9\% \pm 2.7\%$  to  $11.9\% \pm 3.8\%$ , and in seven experiments, there was an increase, from  $25.5\% \pm 2.0\%$  to  $42.3\% \pm 4.3\%$ , when uterine blood flow was reduced. In the seven experiments in which a decrease in the incidence of high-voltage electrocortical activity was observed, there was an increase in the percentage of time spent in intermediate electrocortical activity, from  $4.8\% \pm 1.9\%$  to  $29.1\% \pm 5.9\%$ . When all experiments were considered, there was a significant increase ( $p < 0.02$ ) in the incidence of intermediate electrocortical activity during the first hour of reduced uterine blood flow when compared with 6-hour control recordings. The increase was not different, however, from the percentage of time spent in intermediate electrocortical activity during the 2 hours before the reduction in uterine blood flow.

Fetuses spent 10.7% of the time in transitional electrocortical activity during the 2-hour control period and there was no significant change when uterine blood flow was reduced. There was no change in the number or mean duration of episodes of high-voltage, intermediate, or transitional electrocortical activity during or after the reduction in uterine blood flow (Table II). On the average, changes in sleep states occurred  $8.4 \pm 0.6$  times per hour during the 2-hour control period, and this was not affected by a reduction in uterine blood flow. No correlation was found between the changes in electrocortical activity and changes in  $\text{SaO}_2$ ,  $\text{PO}_2$ ,  $\text{PCO}_2$ , or pH when uterine blood flow was reduced. However, there was a negative correlation between the decrease in the percentage of time spent in low-voltage electrocortical activity and gestational age ( $r = -0.66$ ,  $p < 0.02$ ). During the period of reduced uterine blood flow, there was no significant correlation between gestational age and the incidence of high-voltage electrocortical activity whereas gestational age was inversely correlated with the percentage of time spent in intermediate electrocortical activity ( $r = -0.55$ ,  $p < 0.05$ ).

**Electrooculogram.** During control recordings, the electrooculogram indicated that eye movements were usually present during times of low-voltage electrocortical activity (Fig. 1). When uterine blood flow was reduced, eye movements were abolished or markedly reduced despite the presence of shortened episodes of low-voltage electrocortical activity. After the release of the clamp, the electrooculogram promptly returned to normal activity.

**Breathing movements.** For the analysis of the effects of reduced uterine blood flow on fetal breathing movements, two gestational age groups have been used: group 1 (110 to 121 days' gestation,  $n = 6$ , four fetuses) and group 2 (125 to 141 days' gestation,  $n = 6$ , four fetuses). Fetal breathing movements occurred in group 1 fetuses  $22.9\% \pm 3.5\%$  of the time during the 2-hour control period, decreasing to  $6.4\% \pm 2.7\%$  during the first hour of reduced uterine blood flow ( $p < 0.05$ ). During the second hour of reduced uterine blood flow, however, there was no significant difference in the percentage of time spent breathing when compared with either the 2-hour control period or the equivalent time in control recordings (Fig. 4).

Fetuses in group 2 made fetal breathing movements  $39.7\% \pm 4.6\%$  of the time during the 2-hour control period and this was reduced to  $9.2\% \pm 2.4\%$  ( $p < 0.05$ ) and  $0.5\% \pm 0.3\%$  ( $p < 0.01$ ) of the time during the first and second hours, respectively, of reduced uterine blood flow (Fig. 4). The incidence of fetal breathing movements returned to control values immediately after release of the clamp in both groups. There was no difference in either  $\text{SaO}_2$  or blood gases between experiments in groups 1 and 2 when uterine blood flow was reduced.

On the average, deep inspiratory efforts occurred  $14.0 \pm 2.7$  times per hour in group 1 and  $3.1 \pm 1.0$  times per hour in group 2 ( $p < 0.05$ ). The incidence of deep inspiratory efforts did not change as a result of a reduction in uterine blood flow in either group.

**Skeletal muscle activity.** Integrated electromyographic activity in nuchal and limb muscles declined by approximately 70% as a result of the reduction in uterine blood flow (Fig. 5) and no influence of gestational age was observed. Integrated electromyographic activity from both neck and limb muscles returned to control values immediately after the release of the clamp.

### Comment

In these experiments, we have produced controlled, reversible fetal hypoxemia by mechanically restricting the flow of blood to the gravid uterus in unanesthetized sheep. Although we did not measure uterine blood flow, previous investigators<sup>11</sup> using this technique in pregnant sheep have demonstrated a reduction in blood flow in the main uterine arteries when the common internal iliac artery is compressed. In our experiments, reduced uterine blood flow caused fetal hypoxemia with a mild hypercarbia and acidosis. It is of interest that fetal arterial  $\text{PCO}_2$  was only marginally increased despite a 50% reduction in arterial  $\text{SaO}_2$ . It is possible that the degree of reduction in maternal placental blood flow in these experiments was not great enough to interfere significantly with placental transport of carbon dioxide. Alternatively, carbon dioxide production by the fetus may have decreased as a result

of decreased metabolism. Since the fetal acidosis remained when arterial  $\text{PCO}_2$  had returned to normal levels, a metabolic acidosis must develop when uterine blood flow is restricted sufficient to decrease fetal arterial  $\text{SaO}_2$  to 30%. Clark et al.<sup>13</sup> have reported a two-fold increase in fetal lactate concentrations when uterine blood flow is reduced by 50%, leading to the same degree of fetal hypoxemia as was present in our experiments.

In confirmation of earlier studies of maternal hypoxemia,<sup>5,7</sup> we found that reducing uterine blood flow led to major changes in fetal sleep states. We observed a decrease in the incidence of low-voltage electrocortical activity, which was due to a reduction in the duration of episodes rather than a reduction in their number. This is in agreement with previous investigations<sup>5,7</sup> but conflicts with the results of Adamson et al.<sup>8</sup> The explanation for this variation in the effects of maternal hypoxemia on electrocortical activity is unclear. It has been suggested that the effect of fetal hypoxemia on electrocortical activity may be attributed to associated changes in pH.<sup>8</sup> Although a small degree of acidosis did occur in our experiments, the reduction in fetal arterial  $\text{PO}_2$  was less than that which Natale et al.<sup>7</sup> and Adamson et al.<sup>8</sup> observed but similar to that reported by Boddy et al.<sup>5</sup> Furthermore, we were unable to demonstrate a relationship between any of the observed changes in electrocortical activity and the degree of acidosis. We did, however, observe an inverse correlation between the gestational age of the fetus and the decrease in the incidence of low-voltage electrocortical activity when uterine blood flow was reduced. Thus older fetuses may be more resistant to the effects of hypoxemia on electrocortical activity. It is possible that the conflicting reports on the effects of hypoxia on fetal electrocortical activity may be partly explained by a difference in the gestational ages of the fetuses when studied.

Although we did not observe a consistent increase in the incidence of high-voltage electrocortical activity, fetuses that did not show this increase had an increase in what we have defined as intermediate electrocortical activity. In acute experiments performed in fetal sheep, the electroencephalogram band width of 10 to 13 cycles per second has been shown to be the most sensitive to hypoxia.<sup>14</sup> It would be of interest to perform a power spectral analysis of fetal electrocortical activity when uterine blood flow is reduced, to identify further the effects of prolonged hypoxemia. The electrocortical activity, as recorded from electrodes placed over the surface of the cortex in fetal sheep, is generally considered to arise from cortical neurons, but it is possible that it arises from structures that are deeper within the brain.<sup>15</sup> It has been shown that spontaneous activity of cortical cells in the fetal cerebellum is markedly reduced during maternal hypoxemia.<sup>16</sup> However, the question

of whether hypoxia has a generalized effect on neuronal activity throughout the higher central nervous system centers remains unanswered.

These experiments have confirmed the previously identified inhibitory effect of hypoxemia on fetal breathing movements.<sup>5,6,8,17</sup> All of these previous studies were conducted by having the ewe inspire a reduced oxygen gas mixture for  $\leq 60$  minutes. In agreement with Clewlow et al.,<sup>17</sup> we observed less inhibition of fetal breathing movements in fetuses younger than 121 days than at later gestational ages. During the second hour of reduced uterine blood flow, the incidence of fetal breathing movements was unchanged from values in the control period in younger fetuses, whereas in fetuses greater than 125 days' gestation fetal breathing movements were reduced for the duration of the 2 hours. The mechanisms underlying the inhibition of fetal breathing movements by hypoxemia are unknown although this inhibition is likely due to the activation of inhibitory neural networks within the brain stem.<sup>15</sup> It is possible that these networks are not sufficiently developed in younger fetuses to allow the inhibition of fetal breathing movements to persist during prolonged hypoxemia. The fact that they were initially inhibited, however, suggests that there may be some adaptation to prolonged hypoxemia in younger fetuses. Under normal conditions, in fetuses  $> 120$  days' gestation, fetal breathing movements are present only during times of low-voltage electrocortical activity.<sup>4</sup> The reduction in the incidence of fetal breathing movements, however, cannot be explained by the decrease in low-voltage electrocortical activity since fetal breathing movements are inhibited during these shortened episodes when uterine blood flow is reduced.

Although in these experiments the incidence of fetal breathing movements was low (23%) during the 2-hour control period for fetuses  $< 121$  days' gestation, this may be explained by the observation that changes in tracheal pressure may be difficult to detect in young fetuses despite almost continuous activity of the diaphragm and intercostal muscles.<sup>17</sup> Since the measurement of fetal breathing movements in the human fetus is dependant upon the presence of excursions in either the chest wall or the diaphragm, it is appropriate to have used changes in tracheal pressure to document the incidence of fetal breathing movements in this study. Further experiments are required to determine the effects of prolonged reductions in uterine blood flow on electromyographic activity in the diaphragm and intercostal muscles of fetal sheep.

We observed a marked decrease in electromyographic activity in both nuchal and limb muscles indicating that the inhibitory effect of hypoxemia on skeletal muscle activity is generalized and not confined to specific muscle beds. These results are in agreement with those of Natale et al.,<sup>7</sup> who demonstrated that ma-

ternal hypoxemia leads to a reduction in fetal forelimb movements. We did not find an effect of gestational age on the inhibition of skeletal muscle activity, as was seen with fetal breathing movements.

It has been reported that fetal breathing movements are associated with an increase in fetal oxygen consumption of 17% to 30%.<sup>18,19</sup> By reducing the demands for nonessential activities such as fetal breathing movements and other skeletal muscle activity, the fetus may therefore redirect the available oxygen to the brain, myocardium, and adrenal glands through a redistribution of cardiac output.<sup>20</sup> Recent experiments by Wilkening and Meschia,<sup>21</sup> using graded reductions in uterine blood flow, have shown that oxygen delivery to the sheep fetus must be reduced by 50% before there is a significant fall in fetal oxygen consumption. It is unlikely that the decrease in the percentage of time that the fetus spends in low-voltage electrocortical activity is an attempt by the fetus to conserve oxygen when its delivery is reduced, since the cerebral metabolic rate remains constant despite a decrease in arterial  $PO_2$  to 14 mm Hg.<sup>22</sup> In addition, there was no significant difference in overall fetal oxygen consumption between episodes of low-voltage and high-voltage electrocortical activity.<sup>23</sup> The question of whether fetal oxygen consumption diminished as a result of the decrease in fetal breathing movements and skeletal muscle activity seen in these studies is yet to be determined.

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# Blunted response of maternal ovine placental lactogen levels to arginine stimulation after single umbilical artery ligation in pregnant sheep

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Ovine placental lactogen levels in the maternal circulation are significantly reduced after single umbilical artery ligation in pregnant sheep. We report the ovine placental lactogen response to high-dose amino acid stimulation in four ewes with fetuses that underwent single umbilical artery ligation and six control ewes with fetuses that underwent sham operation. After maternal infusion with 50 gm of arginine in 350 ml of distilled water, mean ovine placental lactogen levels in ewes with fetuses that underwent single umbilical artery ligation increased by 170%, while mean levels in control ewes increased by 294%. Maternal infusions with hypertonic saline solution of osmolality and volume equal to those of the arginine solutions failed to increase maternal ovine placental lactogen levels. Fetal well-being, both during and after the maternal arginine infusions, was confirmed by unchanged fetal arterial blood gases and catecholamines. The ovine placental lactogen levels in the fetal circulation were not altered by maternal arginine infusion. These data suggest that the correlation between maternal ovine placental lactogen levels and functioning placental mass may be enhanced by arginine stimulation. The possible use of this provocation of placental lactogen levels as a test of placental function in clinical practice is discussed. (AM J OBSTET GYNECOL 1986;154:663-6.)

**Key words:** Ovine placental lactogen, arginine stimulation, placental function

In previous studies we demonstrated that single umbilical artery ligation in fetal sheep is followed by infarction of placental cotyledons within the distribution of the ligated artery, intrauterine growth retardation (IUGR), and decreased ovine placental lactogen levels in the maternal circulation.<sup>1,2</sup> This sheep model bears some resemblance to the ill-defined syndrome of placental insufficiency in human pregnancy. Human placental lactogen levels are of some use as a screening test for placental insufficiency, although the predictive power for the diagnosis of IUGR is generally reported at 30% to 50%.<sup>3</sup> Circulating ovine placental lactogen levels, like ovine growth hormone and human growth hormone levels, may be stimulated by high-dose

amino acid infusion.<sup>4-6</sup> Arginine infusion is widely used as a test for human growth hormone secretory capacity in the investigation of pituitary insufficiency.<sup>7</sup>

In this study, we investigated, in the sheep model, the response of maternal ovine placental lactogen levels to arginine stimulation in ewes with fetuses that underwent single umbilical artery ligation and ewes with controls that underwent sham operation. The effect of this intervention on fetal well-being was assessed by serial determination of fetal arterial blood gases and catecholamine levels.

## Methods

Experiments were performed on 10 ewes with singleton pregnancies. Descriptions of the sheep preparations used in this study have been reported elsewhere.<sup>2</sup> Briefly the single umbilical artery ligation procedure was performed at 108 to 114 days' gestation. Ketamine for anesthesia was administered intravenously through an in-dwelling external jugular venous catheter. A fetal hind limb was exteriorized through a hysterotomy incision and the fetal hind limb artery catheterized. The fetus was then delivered to the level of the umbilicus and one umbilical artery was ligated 3 to 5 cm from the fetal abdominal wall. Finally, the fetus was returned to the uterine cavity, the surgical incisions were closed, and the fetal arterial catheter together with a maternal femoral arterial catheter was

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**Table I.** Gestational age (days) at time of infusion studies

<i>Infusion</i>	<i>SUAL*</i>	<i>Control†</i>
Arginine		
n	4	6
Mean	120	121
Range	115-126	116-127
SD	5.2	4.8
Hypertonic saline solution		
n	3	3
Mean	122	121
Range	117-127	115-128
SD	5.0	6.7

\*Ewes with fetuses that underwent single umbilical artery ligation.

†Ewes with fetuses that underwent sham operation.

exteriorized into a pouch attached to the flank of the ewe. The maternal external jugular venous catheter was securely sutured to the skin and covered with an elastic bandage that encircled the neck of the ewe. Ewes used for control experiments were subjected to an identical procedure that included manipulation but not ligation of one umbilical artery. Throughout the remaining duration of gestation, the ewes were housed in metabolic cages, with the environment controlled for temperature and light. Other biophysical and endocrine data from these animals are reported elsewhere.<sup>2</sup>

Arginine solutions for infusion were prepared by dissolving 50 gm of arginine hydrochloride (Calbiochem) in 500 ml of distilled water, with the pH adjusted to 7.4 by the addition of hydrochloric acid and with sterilization by Millipore filtration (0.22  $\mu$ m). Hypertonic saline solutions of osmolality equal to that of the arginine solutions (1100 mosm/L) were prepared by dissolving 12.2 gm of sodium chloride in 500 ml of distilled water, with the pH adjusted to 7.4 by the addition of sodium hydroxide and with sterilization by Millipore filtration. All solutions were prepared immediately before use. Arginine and hypertonic saline infusion experiments were performed at 115 to 128 days' gestation; mean gestational ages at the time of the infusions were comparable in experimental and control ewes (Table I). All fetal sheep survived a minimum of 5 days after the experiments.

Infusion experiments were performed after a minimum of 6 days' recovery from operation. Dietary intake was not altered before the experiments and an effort was made to avoid disturbance of the ewes on the day of study. Fetal well-being was confirmed before commencement of each infusion study by demonstration of normal fetal arterial blood gases. The solutions were infused through the indwelling jugular venous catheter; blood samples for estimation of maternal ovine placental lactogen, fetal ovine placental lactogen, arterial blood gases, and catecholamines were obtained

through the maternal femoral and fetal hind limb arterial catheters. Samples were collected at -30 and 0 minutes and at regular intervals thereafter as shown in Fig. 1. No more than 2.5 ml of fetal blood was removed at each sample collection. The solutions were infused at a constant rate during a 30-minute period. No ewe was infused with the same solution on more than one occasion and a 2-day recovery period was allowed between infusion of different solutions.

The ovine placental lactogen measurements were done by radioimmunoassay as described previously.<sup>2</sup> Catecholamines were determined by radioenzymatic assay according to previously published methods.<sup>8</sup>

Statistical significance of hormonal responses during infusion experiments was assessed by one-way analysis of variance.

## Results

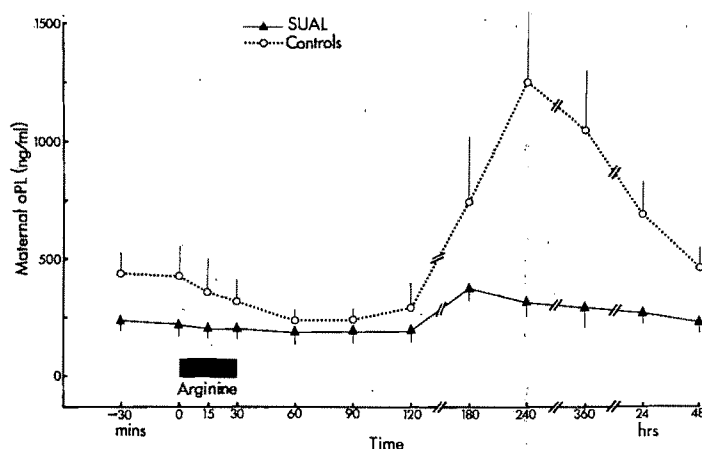
Baseline ovine placental lactogen levels in the maternal circulation were lower in experimental ewes than in control ewes; this observation was consistent with our previous findings.<sup>2</sup> During the initial 30 minutes after infusion with either arginine or hypertonic saline solution, maternal ovine placental lactogen levels decreased slightly. After arginine infusion, mean ovine placental lactogen levels in control ewes increased 294% from a mean baseline value of  $427 \pm 78$  ng/ml ( $\pm$  SEM) to  $1254 \pm 330$  ng/ml at 240 minutes ( $p < 0.001$ ) (Fig. 1). The ovine placental lactogen levels in ewes with experimental fetuses increased 170% from a mean value of  $238 \pm 46$  ng/ml to  $404 \pm 40$  ng/ml at 180 minutes. These alterations in ovine placental lactogen levels after arginine stimulation in ewes with experimental fetuses did not achieve statistical significance.

Maternal ovine placental lactogen levels were not increased by maternal infusion of hypertonic saline solution of volume and osmolality equal to those of the solution used in arginine infusion experiments.

The ovine placental lactogen levels in the fetal circulation were unchanged after arginine infusion in the ewe. As previously reported,<sup>2</sup> ovine placental lactogen levels in experimental fetuses were significantly greater than levels in control fetuses ( $p < 0.005$ ; two-way analysis of variance).

Fetal arterial blood gas values were determined at the time of each fetal blood sample collection. No significant alterations in pH,  $PO_2$ , or  $PCO_2$  were observed during the infusion experiments and all values remained within normal limits.

Arterial norepinephrine and epinephrine levels were also measured in each fetal blood sample. There were no significant alterations in fetal catecholamine levels either during or after maternal arginine infusion in ewes with experimental or control fetuses.



**Fig. 1.** Ovine placental lactogen (oPL) response to arginine infusion in pregnant ewes with fetuses that underwent single umbilical artery ligation (SUAL) ( $n = 4$ ) and control ewes with fetuses that underwent sham operation ( $n = 6$ ). Arginine was infused intravenously from 0 to 30 minutes.

### Comment

Previously we demonstrated IUGR associated with a persistent decrease in ovine placental lactogen levels in the maternal circulation after single umbilical artery ligation.<sup>8</sup> The purpose of this study was to investigate the response of maternal ovine placental lactogen levels to stimulation by arginine infusion in ewes with fetuses that underwent single umbilical artery ligation when compared with the response in control ewes. The mean maternal baseline ovine placental lactogen level before arginine infusion was 238 ng/ml in experimental ewes and 427 ng/ml in control ewes. After arginine infusion, the increase in ovine placental lactogen levels was considerably greater in control animals than in experimental ewes. In control ewes, mean ovine placental lactogen levels reached a maximum of 1256 ng/ml 4 hours after commencement of infusion, while the maximum mean level achieved in ewes with experimental fetuses was 404 ng/ml at 3 hours.

Ovine placental lactogen, ovine growth hormone, and ovine prolactin share similarities in structure and function, and levels of all three hormones in the maternal circulation are increased after arginine infusion.<sup>4,5</sup> In the present study, the magnitude and timing of the ovine placental lactogen response to arginine stimulation in control ewes was similar to the response described by other investigators.<sup>4</sup> However, at present the factors involved in this response are far from clear. Maternal ovine placental lactogen levels also may be stimulated by maternal infusion of ornithine, and ovine placental lactogen levels have been observed to increase in a small number of experiments after infusion with alanine or glycine but not citrulline or glutamic acid.<sup>9,10</sup> Arachidonic acid infusion in pregnant ewes will also stimulate maternal ovine placental lactogen levels; this response cannot be inhibited by prior infusion with

ibuprofen, which suggests that ovine placental lactogen stimulation is regulated by a mechanism other than prostaglandin synthesis.<sup>11</sup> The peak ovine placental lactogen response to each of these stimulating factors occurs 3 to 4 hours after infusion, in contrast to the responses of ovine growth hormone and ovine prolactin, which reach peak levels within 1 to 2 hours of administration of the stimulating infusion.<sup>5</sup> The lack of increase in ovine placental lactogen levels after infusion with hypertonic saline of osmolality and volume equal to those of the arginine infusion solution, observed in both this study and others,<sup>4</sup> indicates that the response to arginine infusion is independent of osmolality per se. However, the hemodilution resulting from infusion of solutions of such high osmolality and volume most likely accounts for the transient decrease in ovine placental lactogen levels observed in the maternal circulation within 1 to 2 hours of infusion.

In clinical practice, stimulation of human growth hormone levels by arginine infusion is widely used to assess pituitary reserve in patients in whom unstimulated human growth hormone levels are within normal limits.<sup>7</sup> In human pregnancy, circulating human placental lactogen levels bear some relation to functioning placental mass,<sup>12</sup> but the sensitivity and specificity of a single low human placental lactogen value in the diagnosis of placental insufficiency and IUGR are low.<sup>3</sup> The results of the present study suggest that arginine stimulation of human placental lactogen levels may be of use as a test of placental function, since a blunted response of maternal ovine placental lactogen levels to this stimulation was observed in ewes with experimental fetuses when compared with the response in control ewes. Serial assessment of the fetal condition by fetal arterial blood gases and catecholamine measurement, both during and after the maternal arginine infusions,

revealed no alteration in fetal well-being as a result of this intervention. The response of human placental lactogen levels to arginine infusion in normal human pregnancy has not been fully investigated. In the few studies reported, no consistent response of human placental lactogen levels to arginine stimulation was found, perhaps because samples were collected for only 90 minutes after infusion, well before the expected rise in human placental lactogen would have occurred.<sup>13-15</sup> Fetal ovine placental lactogen levels, in both this and our previous study,<sup>2</sup> were greater in experimental fetuses than in control fetuses. These fetal ovine placental lactogen levels remained unaltered during and after maternal arginine infusion; this finding is consistent with reports from other investigators<sup>10</sup> and indicates the factors regulating ovine placental lactogen secretion in the mother and fetus are distinct.<sup>2, 10</sup>

In summary, we have demonstrated that the response of ovine placental lactogen levels to arginine infusion in ewes with fetuses that have undergone single umbilical artery ligation is significantly less than the response to infusion in control ewes. Fetal ovine placental lactogen, arterial blood gas, and catecholamine levels were unchanged during and after the maternal arginine infusions. The possible use of arginine stimulation of human placental lactogen levels as a test of placental function in human pregnancy warrants further investigation.

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# The effects of fetal exchange transfusion with a red blood cell substitute

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Isovolemic exchange transfusion in the fetal lamb in utero was performed with the use of Fluosol-DA (20%), a perfluorochemical erythrocyte substitute. With maternal hyperoxygenation, a physiologic  $PO_2$  was maintained in the fetal lambs, although the total fetal oxygen content decreased as the hematocrit dropped. Because of the oxygen-carrying properties of the perfluorochemical emulsion, the fraction of fetal oxygen carried in the dissolved state increased significantly when compared with that in controls that received saline solution. (AM J OBSTET GYNECOL 1986;154:667-74.)

**Key words:** Fetal respiratory physiology, erythrocyte substitute, placental gas transfer

The safety and efficacy of perfluorochemicals in both improving oxygen delivery and maintaining circulation during conditions of acute blood loss have been demonstrated during in vivo studies of both nonpregnant laboratory animals and nonpregnant humans.<sup>1,2</sup> Recently, it was demonstrated that oxygen delivery to the fetal lamb was not impaired under conditions of near-total maternal erythrocyte exchange with Fluosol-DA (20%), an acellular oxygen carrier.<sup>3</sup> Fluosol-DA (20%) is a milky white synthetic emulsion (Table I) consisting of electrolytes, starch, and the emulsified perfluorochemicals perfluorodecalin and perfluorotripropylamine. This report presents the results of a study of fetal cardiorespiratory effects of an isovolemic exchange transfusion of the near-term fetal lamb with Fluosol-DA (20%).

## Material and methods

Four groups of mixed-breed ewes between 135 and 142 days' gestation received ketamine hydrochloride (1 mg/kg intramuscularly) premedication and then were anesthetized with ketamine by continuous intravenous drip at 7 to 9 mg/min after cut-down cannulation of a jugular vein. Each fetus then underwent isovolemic exchange transfusion, according to group, with either Fluosol-DA (20%) or normal saline solution, during which its mother breathed either room air or 100%

**Table I.** Fluosol-DA (20%) emulsion composition after reconstitution

<i>Ingredient</i>	<i>Quantity (gm/dl)</i>
Perfluorodecalin	14.0
Perfluorotripropylamine	6.0
Hydroxyethyl starch	3.0
Pluoronic F-68	2.7
Glycerol	0.8
Sodium chloride	0.6
Egg yolk phospholipids	0.4
Sodium bicarbonate	0.21
Glucose	0.18
Oleic acid	0.04
Potassium chloride	0.03
Calcium chloride	0.02
Magnesium chloride	0.02
Water for injection	q.s.

oxygen at 1 atmosphere pressure. Group I ( $n = 3$ ) and group II ( $n = 3$ ) ewes breathed room air throughout the procedure, while ewes of groups III ( $n = 3$ ) and IV ( $n = 2$ ) breathed near-100% oxygen via a rebreathing bag inflated with 100% oxygen at a flow rate of greater than 6 L/min. Groups I and III fetal lambs underwent continuous isovolemic exchange transfusion of Fluosol-DA (20%) for whole blood via the jugular veins; groups II and IV lambs similarly underwent exchange of a normal saline solution for whole blood.

A maternal femoral artery was catheterized to permit periodic blood collection and continuous systemic arterial pressure measurement with transducers (Hewlett-Packard Model 1280) and recording on a multichannel data recorder (Hewlett-Packard Model 7788A). After a laparotomy incision, a catheter was placed into a uterine vein draining the pregnant uterine horn to permit periodic sampling. The fetal head was then exteriorized with the amnion intact through a hysterotomy incision. Indwelling catheters were placed in each fetal jugular vein to permit exchange transfusion.

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**Table II.** Cardiorespiratory effects in group I of fetal exchange transfusion with fluosol-DA (20%)

	<i>Fetal</i>						
	<i>Hematocrit (%)</i>	<i>Fluorocrit (%)</i>	<i>Arterial PO<sub>2</sub> (torr)</i>	<i>Arterial (pH)</i>	<i>Arterial PCO<sub>2</sub> (torr)</i>	<i>f [O<sub>2</sub>] <sub>dis</sub><sup>†</sup></i>	<i>f [O<sub>2</sub>] <sub>WB</sub><sup>‡</sup></i>
Baseline room air	41.5 ± 3.6	0	23.3 ± 0.2	7.376 ± 0.034	38.8 ± 4.1	0.074 ± 0.0C1	9.052 ± 0.805
15 min	37.5 ± 0.4	1.8 ± 0.8	19.5 ± 0.7	7.366 ± 0.047	42.1 ± 4.1	0.072 ± 0.0C2	6.588 ± 0.056
30 min	34.2 ± 0.2	3.7 ± 0.6	19.5 ± 1.2	7.363 ± 0.035	40.3 ± 6.9	0.082 ± 0.0C5	5.978 ± 0.059
45 min	30.0 ± 3.6	5.3 ± 1.2	19.8 ± 1.5	7.342 ± 0.018	40.5 ± 6.7	0.092 ± 0.0C7	5.421 ± 0.666
60 min	27.0 ± 2.9	6.3 ± 1.9	19.5 ± 2.0	7.307 ± 0.019	44.7 ± 4.8	0.097 ± 0.010	4.760 ± 0.492
75 min	25.0 ± 1.4	7.0 ± 1.7	19.0 ± 1.6	7.265 ± 0.046	47.5 ± 4.8	0.098 ± 0.010	4.331 ± 0.270
90 min	23.0 ± 2.2	8.3 ± 1.2	18.5 ± 1.4	7.212 ± 0.083	47.3 ± 8.5	0.013 ± 0.0C8	3.871 ± 0.364
105 min	20.7 ± 2.6	9.3 ± 1.2	19.5 ± 2.5	7.182 ± 0.100	50.3 ± 6.9	0.114 ± 0.014	3.705 ± 0.443

\*Volume percentage of perfluorochemicals centrifuged from blood.

†Milliliters of oxygen per deciliter; oxygen content dissolved in aqueous and perfluorochemical phases, fetal arterial blood.

‡Milliliters of oxygen per deciliter; total oxygen content, fetal arterial whole blood.

**Table III.** Cardiorespiratory effects in group II of fetal exchange transfusion with normal saline solution

	<i>Fetal</i>						<i>Maternal arterial PO<sub>2</sub> (torr)</i>
	<i>Hematocrit (%)</i>	<i>Arterial PO<sub>2</sub> (torr)</i>	<i>Arterial (pH)</i>	<i>Arterial PCO<sub>2</sub> (torr)</i>	<i>f [O<sub>2</sub>] <sub>dis</sub><sup>*</sup></i>	<i>f [O<sub>2</sub>] <sub>WB</sub><sup>†</sup></i>	
Baseline room air	44.7 ± 7.4	19.0 ± 0.2	7.306 ± 0.071	44.7 ± 3.5	0.060 ± 0.001	7.392 ± 1.323	58.5 ± 7.5
15 min	43.3 ± 7.0	17.9 ± 1.1	7.309 ± 0.069	46.4 ± 3.8	0.057 ± 0.003	6.435 ± 1.051	61.6 ± 7.0
30 min	41.1 ± 6.8	19.3 ± 1.1	7.276 ± 0.089	44.6 ± 3.9	0.061 ± 0.003	7.012 ± 1.153	62.2 ± 7.8
45 min	38.8 ± 7.9	20.0 ± 0.6	7.206 ± 0.094	47.1 ± 5.5	0.063 ± 0.002	6.931 ± 1.376	64.7 ± 8.0
60 min	35.3 ± 7.6	19.8 ± 1.0	7.131 ± 0.117	50.0 ± 5.8	0.063 ± 0.003	6.326 ± 1.377	67.1 ± 8.5
75 min	31.7 ± 8.0	19.2 ± 0.2	7.004 ± 0.188	56.3 ± 10.7	0.061 ± 0.001	5.392 ± 1.360	63.3 ± 8.4
90 min	34.3 ± 6.8	17.9 ± 0.1	7.018 ± 0.218	62.1 ± 19.8	0.057 ± 0.001	5.069 ± 1.003	60.2 ± 10.2
105 min	30.5 ± 7.5	20.2 ± 2.3	6.999 ± 0.199	64.4 ± 19.1	0.064 ± 0.007	5.448 ± 1.326	54.0

\*Milliliters of oxygen per deciliter; physically dissolved oxygen, fetal arterial blood.

†Milliliters of oxygen per deciliter; total oxygen content, fetal arterial whole blood.

An indwelling catheter was likewise placed a distance of 4 to 8 cm into a fetal carotid artery to allow periodic sampling and continuous pressure recording.

After a postoperative stabilization period, exchange was accomplished via continuous infusion-withdrawal pumps at a rate of 5 to 10 ml/min during 75 to 105 minutes. Fluosol-DA (20%) or normal saline solution (391 ± 67 ml, 81 to 117 ml/kg) was infused, and whole blood (272 ± 48 ml, 56 to 80 ml/kg) was removed. The target level of exchange was to achieve a fluorocrit of >6% and/or a reduction of hematocrit to half the original level. After these levels were reached or approximated, groups I and II ewes and lambs were put to death by intravenous injection of each with potassium chloride, while groups III and IV ewes were given 100% nitrogen to breathe.

Data collected included continuous maternal femoral and fetal carotid arterial pressure, periodic (every 15 minutes) blood gases and pH from maternal and fetal arteries and from the maternal uterine vein, maternal hematocrits, and fetal hematocrits and fluorocrits. Hematocrits and fluorocrits (the volume percentage of the white bottom layer of perfluorochemical centrifuged

from the blood) were determined in capillary tubes that were centrifuged for 5 minutes at 10,000 rpm. Samples of blood for analysis of gases and pH were obtained simultaneously and drawn anaerobically. pH and the partial pressures of oxygen (PO<sub>2</sub>) and carbon dioxide (PCO<sub>2</sub>) were measured with an Instrument Laboratory No. 113 gas analyzer at 38° C.

The total oxygen content of maternal arterial whole blood, M[O<sub>2</sub>]<sub>WB</sub>, and uterine venous blood, M[O<sub>2</sub>]<sub>WB</sub><sup>uv</sup>, was calculated by adding the oxygen bound to hemoglobin to the oxygen in physical solution (M[O<sub>2</sub>]<sub>WB</sub> = [O<sub>2</sub>]<sub>rbc</sub> + [O<sub>2</sub>]<sub>dis</sub>), expressed in milliliters of oxygen per deciliter. The [O<sub>2</sub>]<sub>rbc</sub> was calculated by multiplying the hemoglobin concentration, the derived percentage of oxygen saturation from the sheep blood oxygen dissociation curve [O<sub>2</sub>]<sub>dis</sub>, and the Hüfner coefficient (1.34 ml of oxygen per gram of hemoglobin per 100 ml). [O<sub>2</sub>]<sub>dis</sub> was calculated as the product of the partial pressure of oxygen and the Bunsen solubility coefficient of oxygen in plasma (0.0000316 × PO<sub>2</sub> × 100).

Total oxygen content of fetal arterial blood in groups II and IV was calculated in an identical fashion, albeit with the use of the oxygen dissociation curve for fetal

Maternal		
Uterine vein PO <sub>2</sub> (torr)	Arterial PO <sub>2</sub> (torr)	Uterine vein PCO <sub>2</sub> (torr)
42.9 ± 1.9	59.4 ± 15.3	35.4 ± 2.6
40.7 ± 1.8	71.8 ± 2.0	33.2 ± 2.7
41.1 ± 0.8	77.1 ± 4.8	32.9 ± 2.5
40.7 ± 2.1	75.9 ± 5.2	32.0 ± 5.8
39.7 ± 1.5	71.1 ± 5.6	36.2 ± 2.6
39.8 ± 2.0	70.3 ± 4.0	34.2 ± 1.8
40.6 ± 1.2	71.5 ± 2.7	35.4 ± 0.6
42.5 ± 3.3	78.3 ± 4.0	33.7 ± 0.9

lamb blood. Since the blood of groups I and III lambs had a third oxygen-carrying phase (the perfluorochemical emulsion), total oxygen content was determined with the use of the calculation of the whole blood-perfluorochemical emulsion mixture oxygen content by means of Henry's law (volume = pressure × solubility coefficient) applied to the perfluorochemical and aqueous phases of the whole blood mixture.<sup>4</sup> Oxygen carried by the perfluorochemical phase,  $f[O_2]_{pfc}$ , was determined by the formula  $[O_2]_{pfc} = \alpha_{pfc} \times \frac{PO_2}{760} \times \text{fluorocrit} \times 0.000318 \times PO_2 \times \text{fluorocrit}$ .

Oxygen carried in the aqueous phase,  $[O_2]_{aqu}$ , was determined by the formula  $[O_2]_{aqu} = \alpha_{aqu} \times \frac{PO_2}{760} \times (100 - \text{fluorocrit}) = 0.0000316 \times PO_2 \times (100 - \text{fluorocrit})$ . These two quantities together compose the dissolved phase,  $f[O_2]_{diss} = f[O_2]_{pfc} + f[O_2]_{aqu}$ , and thus the total oxygen content of fetal arterial blood during exchange with Fluosol-DA (20%) is  $f[O_2]_{WB} = f[O_2]_{rbc} + f[O_2]_{diss} = f[O_2]_{rbc} + f[O_2]_{pfc} + f[O_2]_{aqu}$ .

Fluosol-DA (20%) was supplied by Alpha Therapeutic Corporation, Los Angeles, California, as manufactured by Green Cross Corporation, Osaka, Japan. The contents of the perfluorochemical emulsion are listed in Table I. It has the appearance and consistency of skim milk, a pH of 7.40, an osmolarity of 410 mOsm, oncotic pressure of 380 to 395 mm H<sub>2</sub>O, and the average particle size of the perfluorochemical compound is 0.2 μm.

The experimental protocol consisted of: (1) a post-operative stabilization period of 15 to 30 minutes, during which the ewes of all groups breathed room air and during which maternal and fetal arterial pressures were recorded continuously, with intermittent determinations of blood gases, pH, and hematocrit from the fetal carotid artery and maternal femoral artery and uterine vein; (2) a second stabilization phase during which groups III and IV ewes breathed 100% oxygen and during which similar recording and sampling were accomplished; (3) isovolemic fetal exchange of Fluosol-DA (20%) (groups I and III) or normal saline solution

(groups II and IV) for whole blood until fluorocrits of >6% and/or hematocrits of approximately half their original values were achieved; (4) continuous recording of maternal and fetal arterial blood pressure; (5) periodic (every 15 minutes during exchange) determinations of maternal arterial and uterine vein blood gases, pH, and hematocrit and fetal arterial blood gases, pH, hematocrit, and fluorocrit.

## Results

Relevant cardiorespiratory data are listed in Tables II to V. In groups I and III (the Fluosol-DA (20%)—exchanged lambs), fetal lambs weighed  $4031 \pm 844$  gm, and their calculated blood volume was  $363 \pm 76$  ml. Therefore, each fetus underwent a 74% exchange with Fluosol-DA (20%) during 75 to 105 minutes. Base-line hematocrits were  $41.1\% \pm 2.7\%$ , and by the end of the exchange period hematocrits had decreased to  $21.3\% \pm 5.4\%$ , a drop to 52% of their original levels. Maximum fluorocrits achieved were  $8.4\% \pm 1.6\%$ .

The fetuses in groups II and IV, which received normal saline solution, weighed  $4155 \pm 672$  gm. The calculated blood volume was  $374 \pm 61$  ml, and they underwent 80% exchange. The hematocrits dropped from  $41.4\% \pm 7.2\%$  to  $23.9\% \pm 1.7\%$ , 58% of the original levels. Fetal and maternal arterial pressures remained stable during the test period in all preparations.

**Oxygen.** In the lambs that underwent exchange transfusion with Fluosol-DA (20%), fetal arterial PO<sub>2</sub> was able to be maintained at physiologic levels ( $30.9 \pm 3.3$  torr) (Fig. 1) under conditions of maternal hyperoxygenation, even as the fetal hematocrit decreased almost to half of its original value. Moreover, in these group III fetuses, the fraction of total fetal oxygen in the dissolved state ( $f[O_2]_{diss}$ ) increased significantly relative to that in controls (Fig. 2) (Student's *t* distribution, one-sided,  $\alpha = 0.05$ ).

**Carbon dioxide.** Under the conditions of the study, the PCO<sub>2</sub> of fetal arterial blood in all four groups increased significantly from  $46.5 \pm 5.2$  to  $62.0 \pm 7.4$  torr during the exchange, and the pH of fetal arterial blood decreased from  $7.305 \pm 0.046$  to  $7.084 \pm 0.081$ . There was no difference in this behavior between the Fluosol-DA (20%)—exchanged groups and the saline solution—exchanged groups, nor did maternal hyperoxygenation make a difference. These trends began early in the exchange and persisted throughout.

## Comment

Perfluorochemical emulsions used as red blood cell substitutes have been shown in clinical use to directly increase the delivery of oxygen to tissues by maintaining perfusion and transporting oxygen and carbon dioxide.<sup>1,2</sup> Fluosol-DA (20%) transports oxygen and carbon dioxide by direct solubility, and the volumes of

**Table IV.** Cardiorespiratory effects in group III of fetal exchange transfusion with fluosol-DA (20%)

	Fetal						
	Hematocrit (%)	Fluorocrit* (%)	Arterial PO <sub>2</sub> (torr)	Arterial (pH)	Arterial PCO <sub>2</sub> (torr)	f[O <sub>2</sub> ] <sub>dis</sub> †	f[O <sub>2</sub> ] <sub>wb</sub> ‡
Baseline room air	40.7 ± 1.2	0	24.1 ± 2.8	7.248 ± 0.049	49.8 ± 1.0	0.076 ± 0.009	9.380 ± 0.293
100% oxygen	40.7 ± 1.7	0	36.4 ± 5.5	7.205 ± 0.034	52.6 ± 2.7	0.115 ± 0.017	14.509 ± 0.566
15 min	35.7 ± 0.8	1.7 ± 0.6	34.6 ± 5.3	7.195 ± 0.024	56.1 ± 3.5	0.126 ± 0.019	12.454 ± 0.341
30 min	32.7 ± 1.3	3.0 ± 0.4	29.3 ± 2.9	7.184 ± 0.011	58.4 ± 4.6	0.118 ± 0.012	9.967 ± 0.387
45 min	28.1 ± 1.3	3.8 ± 0.5	28.6 ± 2.2	7.184 ± 0.025	60.8 ± 3.8	0.122 ± 0.010	8.414 ± 0.372
60 min	26.3 ± 2.1	5.2 ± 0.8	28.1 ± 1.8	7.167 ± 0.032	62.9 ± 5.2	0.130 ± 0.008	7.647 ± 0.627
75 min	24.5 ± 1.5	7.2 ± 0.8	28.3 ± 2.3	7.146 ± 0.046	62.5 ± 4.5	0.137 ± 0.011	7.230 ± 0.460

\*Volume percentage of perfluorochemicals centrifuged from blood.

†Milliliters of oxygen per deciliter; oxygen content dissolved in aqueous and perfluorochemical phases, fetal arterial blood.

‡Milliliters of oxygen per deciliter; total oxygen content, fetal arterial whole blood.

**Table V.** Cardiorespiratory effects in group IV of fetal exchange transfusion with normal saline solution

Fetal	Fetal					
	Hematocrit (%)	Arterial PO <sub>2</sub> (torr)	Arterial (pH)	Arterial PCO <sub>2</sub> (torr)	f[O <sub>2</sub> ] <sub>dis</sub> *	f[O <sub>2</sub> ] <sub>wb</sub> †
Baseline room air	36.5 ± 3.0	22.4 ± 0.6	7.288 ± 0.013	52.5 ± 1.5	0.071 ± 0.002	7.661 ± 0.645
100% oxygen	36.3 ± 2.8	29.5 ± 1.5	7.256 ± 0.024	53.5 ± 0.1	0.093 ± 0.005	11.068 ± 0.943
15 min	34.0 ± 3.0	28.1 ± 0.9	7.242 ± 0.038	56.5 ± 2.0	0.089 ± 0.003	9.817 ± 0.887
30 min	32.8 ± 2.8	27.2 ± 1.2	7.240 ± 0.033	58.0 ± 1.6	0.086 ± 0.004	9.035 ± 0.848
45 min	30.5 ± 1.5	26.7 ± 0.7	7.221 ± 0.019	57.3 ± 1.8	0.084 ± 0.002	8.226 ± 0.417
60 min	28.8 ± 1.3	25.6 ± 1.4	7.191 ± 0.030	57.3 ± 3.8	0.081 ± 0.004	7.434 ± 0.320
75 min	26.3 ± 1.8	25.7 ± 2.4	7.137 ± 0.003	59.3 ± 0.8	0.081 ± 0.007	6.915 ± 0.489
90 min	23.5 ± 1.0	24.5 ± 2.3	7.073 ± 0.003	71.5 ± 1.0	0.077 ± 0.007	5.678 ± 0.228
105 min	23.5 ± 1.0	23.9 ± 3.2	7.010 ± 0.010	70.6 ± 0.6	0.076 ± 0.010	5.168 ± 0.211

\*Milliliters of oxygen per deciliter; physically dissolved oxygen, fetal arterial blood.

†Milliliters of oxygen per deciliter; total oxygen content, fetal arterial whole blood.

the gases vary linearly with their partial pressures, according to Henry's law. Physical considerations limit the maximum concentrations of perfluorochemicals contained in the emulsion. The fluorocrit of Fluosol-DA (20%) has been measured as 14.63% ± 0.06%; in our study we were able to achieve a fluorocrit of 8.4% ± 1.6%, which is 57% of the fluorocrit of the pure emulsion, uncontaminated by blood. At oxygen tensions achieved by the adult breathing room air, Fluosol-DA (20%) has only a limited capacity to carry oxygen and serves primarily as a volume expander. At PO<sub>2</sub>s achieved by breathing 100% oxygen, however, Fluosol-DA (20%) can carry 5.6 ml of oxygen per deciliter, approximately one third that of whole blood. In the presence of a whole blood-Fluosol-DA (20%) mixture, oxygen will be carried in three phases: chemically combined with hemoglobin, dissolved in plasma, and dissolved in the perfluorochemical emulsion. Methods for calculating these volumes, as well as for assessing their delivery and consumption have been described.<sup>4</sup> The tissues will obtain most of their oxygen from the dissolved phase before any is provided by hemoglobin.

The low viscosity of the emulsion and small size of the fluorocarbon particles (0.2 µm) support flow through small or constricted vessels, thus improving delivery to the tissues.<sup>2</sup>

**Oxygen.** Since the classic work of Huggett<sup>5</sup> in 1927, it has been accepted that oxygen crosses the placental membrane by a process of simple diffusion along a concentration gradient; normal PO<sub>2</sub> values of 27 torr for the umbilical vein and 15 torr for the umbilical artery have been calculated by Longo. Further work by a number of investigators has shown that the rate and volume of oxygen transfer across the placenta are affected by many factors, including differences in maternal and fetal PO<sub>2</sub>, maternal and fetal intervillous blood flow, placental permeability, and differences in maternal and fetal PCO<sub>2</sub>.<sup>6</sup> Moreover, during pregnancy, intraerythrocytic concentrations of 2, 3-diphosphoglycerate increase by 30%, causing a shift of the oxygen dissociation curve to the right and enhancing the release of oxygen across the placenta to the fetus.

Fetal oxygen uptake is favored by a high concentration of hemoglobin. Moreover, Battaglia et al.<sup>7</sup> dem-

Maternal		
Uterine vein $PO_2$ (torr)	Arterial $PO_2$ (torr)	Uterine vein $PCO_2$ (torr)
48.4 ± 7.9	69.5 ± 9.1	43.5 ± 2.2
82.9 ± 19.3	334.3 ± 59.3	47.6 ± 0.6
115.1 ± 34.7	306.7 ± 63.3	44.0 ± 5.3
88.3 ± 27.7	296.0 ± 48.0	51.6 ± 3.9
91.0 ± 31.0	298.0 ± 36.0	49.5 ± 0.8
86.7 ± 26.7	311.0 ± 39.0	50.1 ± 2.3
64.6 ± 1.6	211.0 ± 69.0	52.6 ± 4.2

Maternal		
Uterine vein $PO_2$ (torr)	Arterial $PO_2$ (torr)	Uterine vein $PCO_2$ (torr)
65.0	73.5	40.0
51.5	337.5 ± 7.5	48.3
61.0	320.0 ± 96.2	49.5
64.9	320.0 ± 5.0	47.5
52.8	302.5 ± 17.5	44.8
44.0	304.5 ± 7.5	50.0
47.0	302.5 ± 17.5	52.5
52.3	300.0 ± 1.0	52.3
75.8	298.0 ± 8.0	53.0

onstrated that fetal erythrocytes have a much higher affinity for oxygen than adult red blood cells. Fetal hemoglobin can thus be saturated with oxygen at lower partial pressures than adult hemoglobin. Under normal conditions the transfer of oxygen is not thought to be limited by resistance to diffusion at the placental membrane but is flow limited. The force that drives oxygen across the placenta is the gradient in the partial pressure of the physically dissolved gas between maternal and fetal blood.<sup>3</sup> When the mother is breathing room air, according to Henry's law, 0.3 ml of oxygen per deciliter is physically dissolved in the plasma, and 15 to 17 ml of oxygen per deciliter is bound to the hemoglobin. As the physically dissolved oxygen diffuses across the placenta, additional oxygen is released from the maternal hemoglobin. On the fetal side, the opposite occurs: After diffusion across the placenta in the dissolved phase, the gas enters the fetal circulation where it is quickly absorbed by the fetal hemoglobin with its high oxygen affinity.

The oxyhemoglobin dissociation curves of human maternal and fetal blood at pH = 7.4 illustrate that at a given oxygen tension, fetal hemoglobin will be significantly more saturated with oxygen than adult he-

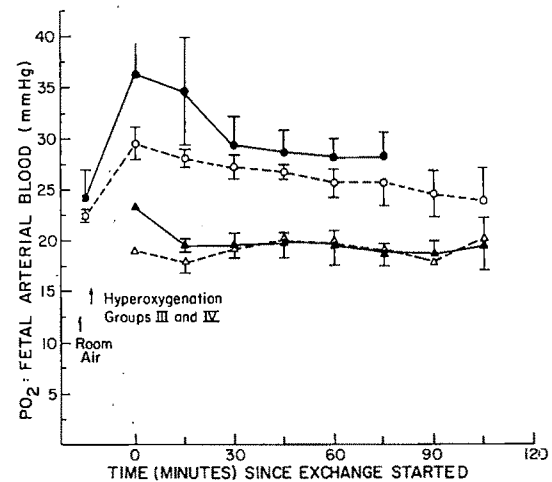


Fig. 1. Partial pressure of oxygen in fetal arterial blood in group I (room air, Fluosol-exchanged) lambs (▲—▲—▲), group II (room air, saline solution-exchanged) lambs (△—△—△), group III (hyperoxygenation, Fluosol-exchanged) lambs (●—●—●), and group IV (hyperoxygenation, saline solution-exchanged) lambs (○—○—○).

moglobin, and the position and steepness of the fetal curve at usual fetal oxygen tensions demonstrate that the oxygen content of fetal blood may be greatly affected by small changes of fetal  $PO_2$ . Yet it has been demonstrated that major increases in maternal arterial oxygen tension have resulted in only modest elevations of fetal  $PO_2$ . Only by hyperbaric oxygen administration to the mother, as shown by Assali et al.,<sup>8</sup> can fetal  $PO_2$ s in excess of 100 mm Hg be achieved. Meschia<sup>9</sup> explained that the large  $PO_2$  differences between maternal arterial blood and umbilical venous blood is primarily the result of the structural characteristics of the placenta and oxygen consumption by the placenta; flow, consequently, resembles that of a concurrent system, in which the oxygen tension of fetal blood at the placental interface may not exceed that of the venous side of the donor stream. Whereas this results in fetal blood having a relatively low oxygen tension, umbilical venous and arterial blood normally contain large amounts of oxygen, because of the high affinity of fetal erythrocytes for oxygen. Because of this, for example, an increase of 5 torr in the arterial  $PO_2$  of the fetus may increase its total blood oxygen content as much as a 500 torr increase in the arterial  $PO_2$  of the mother.<sup>9,10</sup>

Under conditions of maternal hyperoxygenation, perfluorochemical emulsion-exchanged fetuses (group III) did not demonstrate a greater affinity for oxygen than saline solution-exchanged controls (group IV) in that there was no significant increase in total oxygen content in the perfluorochemical emulsion-exchanged fetuses; they were apparently unable to attract increased quantities of oxygen across the placenta (Fig. 3). The group IV fetuses (saline solution, hyperoxy-



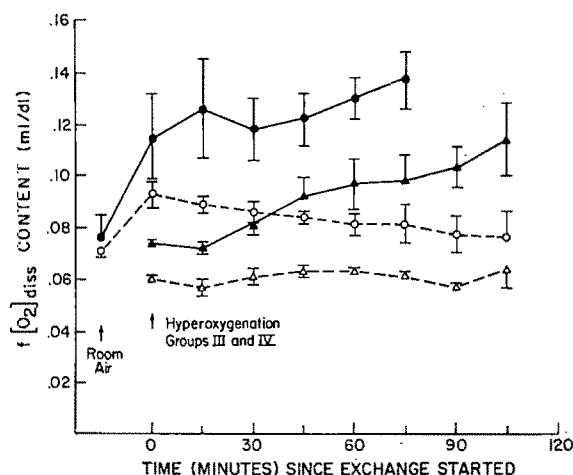


Fig. 2. Oxygen content dissolved in a aqueous and perfluorochemical phases, fetal arterial blood, in group I (room air, Fluosol-exchanged) lambs ( $\blacktriangle$ — $\blacktriangle$ — $\blacktriangle$ ), group II (room air, saline solution-exchanged) lambs ( $\triangle$ — $\triangle$ — $\triangle$ ), group III (hyperoxygenation, Fluosol-exchanged) lambs ( $\bullet$ — $\bullet$ — $\bullet$ ), and group IV (hyperoxygenation, saline solution-exchanged) lambs ( $\circ$ — $\circ$ — $\circ$ ).

genation) had a small apparent but not significant decrease in  $PO_2$  relative to the perfluorochemical emulsion-exchanged fetal lambs (Fig. 1). The group III ewes (perfluorochemical emulsion, hyperoxygenation) likewise had an apparent but not significant increase in maternal uterine vein  $PO_2$  compared to the mothers of the saline solution-exchanged fetuses. When these values were combined, however, the approximate  $PO_2$  gradient, that is, maternal uterine vein  $PO_2$  minus fetal arterial  $PO_2$ , was significantly lower in the saline solution-exchanged preparations than in those in which a Fluosol-DA (20%) exchange was performed. The fact that we were unable to raise fetal arterial  $PO_2$  levels above maternal uterine vein  $PO_2$  levels in spite of the high affinity of the perfluorochemicals for oxygen and even though uterine vein  $PO_2$  levels in the perfluorochemical emulsion-exchanged group appeared to be higher than in the saline solution-exchanged group, is in agreement with the findings of Meschia and lends further support to his theory of concurrent flow.<sup>9, 10</sup> The fetal arterial oxygen tension, on the other hand, was able to be maintained at physiologic levels during the hyperoxygenation tests with induced anemia; the perfluorochemical emulsion-exchanged fetal lambs'  $PO_2$  levels were apparently but not significantly higher than those exchanged with saline solution (Fig. 1).

Barcroft<sup>11</sup> measured the oxygen consumption of the fetal lamb as  $4.8 \pm 0.6$  ml of oxygen per kilogram per minute; Meschia accurately measured umbilical blood flow at approximately 100 ml/kg/minute. With use of these data, one can calculate a critical oxygen content of fetal umbilical blood of  $4.8 \pm 0.6$  ml of oxygen per

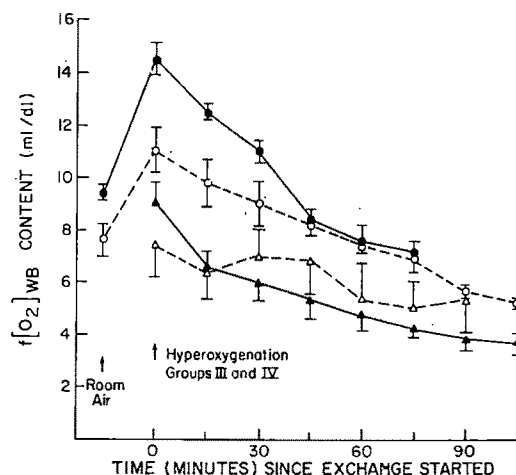


Fig. 3. Total oxygen content of fetal whole blood in group I (room air, Fluosol-exchanged) lambs ( $\blacktriangle$ — $\blacktriangle$ — $\blacktriangle$ ), group II (room air, saline solution-exchanged) lambs ( $\triangle$ — $\triangle$ — $\triangle$ ), group III (hyperoxygenation, Fluosol-exchanged) lambs ( $\bullet$ — $\bullet$ — $\bullet$ ), and group IV (hyperoxygenation, saline solution-exchanged) lambs ( $\circ$ — $\circ$ — $\circ$ ).

deciliter, below which the oxygen needs of the fetus, at least in theory, will not be met. In our study, the fetal lambs of groups I, II, and IV all approached or dropped below that critical level (Fig. 3). In the group III fetuses (perfluorochemical emulsion, hyperoxygenation), however, even in the presence of the physiologic but relatively low  $PO_2$  available on the fetal side of the placenta, there was apparently more oxygen available to fetal tissues than in the saline solution-exchanged controls. In these fetal lambs, as a level of half the original hematocrit was approached, the  $f[O_2]_{WB}$  was still  $7.23 \pm 0.46$  ml of oxygen per deciliter—well above the critical level of 4.2 to 5.4 ml/dl theoretically required to provide sufficient oxygen for the lamb's physiologic needs. If the hematocrit were to continue to decrease, however, the  $f[O_2]_{WB}$  would eventually reach this critical level, because of the nature of the Fluosol-DA (20%), whose oxygen-carrying properties, albeit having 10 times the oxygen-carrying capacity of plasma per volume percent, depend on a high oxygen tension. With use of the calculations of Rosen, a fetus with absolutely no hemoglobin completely exchanged with Fluosol-DA (20%) would be able to achieve a  $f[O_2]_{WB}$  of 4.8 ml of oxygen per deciliter, but to do so would require an umbilical vein  $PO_2$  of more than 600 torr, a level reached experimentally only under hyperbaric conditions. At modest oxygen tensions, such as the 27 torr usually seen in the umbilical vein, perfluorochemicals have only a small capacity to carry oxygen, and the emulsion acts primarily as a volume expander.<sup>4</sup> On the other hand, achieving a fluorocrit of 8% to 10% in an anemic fetus with a  $PO_2$  of 35 to 40 torr and a he-

matocrit of 10% to 15% might provide the additional oxygen necessary to allow survival, especially considering that the small size of the perfluorochemical emulsion particle allows it to deliver oxygen to tissues that might otherwise be inaccessible because of vasospasm.

**Carbon dioxide.** Carbon dioxide crosses the placenta by simple diffusion along a gradient mainly as dissolved carbon dioxide rather than as bicarbonate, as shown by Longo, Hill, and others.<sup>12, 13</sup> The rate and volume of carbon dioxide exchange across the placenta is influenced by umbilical and uterine artery  $PO_2$  values, umbilical and uterine blood flow rates, the hemoglobin buffering capacity, and the hemoglobin concentration.<sup>12, 13</sup> Under normal conditions the  $PO_2$  on the fetal side (43 and 48 mm Hg, respectively, for the umbilical vein and artery)<sup>10</sup> is greater than that on the maternal side, probably because of anatomic and physiologic shunting as well as placental production of  $CO_2$ .

The ability of perfluorochemical emulsions to transport carbon dioxide has been amply demonstrated. In vitro, carbon dioxide is extremely soluble in perfluorochemical emulsions, more so than any other gas. The easy uptake and loss of carbon dioxide by Fluosol-DA (20%) facilitates exchange with the surrounding environment, and depends only on the partial pressure of the gas.<sup>14</sup> During studies involving massively bled dogs which then received perfluorochemical emulsion transfusions, it was established that perfluorochemical emulsions were capable of carrying carbon dioxide as well as oxygen in vivo.<sup>15</sup> The rate of carbon dioxide uptake and release in perfluorochemical particles was investigated kinetically with use of stopped flow spectrophotometry; it took only a few milliseconds for the particles to complete carbon dioxide transfer.<sup>16</sup>

The pH of fetal arterial blood in most mammalian species, including the sheep and the human, is about 0.1 less and the  $PCO_2$  10 to 15 torr higher than that of maternal arterial blood. Nevertheless, a relative respiratory acidosis was observed in the fetal lambs in this study, and various etiologic possibilities were entertained to explain it. It is very unlikely that the increase in  $PCO_2$  and decrease in pH observed was due to interference with carbonic anhydrase activity, as it has been determined that this is not influenced by Fluosol-DA (20%). It was hypothesized that the increased  $PCO_2$  observed in the perfluorochemical emulsion-exchanged fetuses might be due to an excess of bicarbonate in the annex solution, which contains 0.21 gm/dl of sodium bicarbonate. However, it has been shown that bicarbonate is a necessary component of erythrocyte substitute preparations; in its absence, the pH decreases and the carbon dioxide increases to an extent dependent on the quantity of hemoglobin remaining in the circulation.<sup>14</sup>

Cefalo et al.<sup>3</sup> showed that fetal pH and  $PCO_2$  did not

change during maternal exchange transfusion with Fluosol-DA (20%) in intubated ewes breathing 100% oxygen in a carbon dioxide-absorbing system, which suggests that perfluorochemicals on the maternal side of the placenta represent no barrier to carbon dioxide diffusion. In the current study, however, it is apparent that at least part of the observed fetal hypercapnia and acidosis in groups III and IV was related to the significant maternal hypercapnia that was observed, in spite of high oxygen flow rates through the rebreathing bags. The mean maternal arterial and uterine vein  $PCO_2$  values were 32.9 and 34.0 torr, respectively, in the ewes which breathed room air and 45.9 and 48.6 torr, respectively, in the ewes using rebreathing bags. The ewes in groups III and IV, therefore, spent the study period in a state of partially compensated respiratory acidosis caused by their rebreathing significant amounts of their expired carbon dioxide, and this was obviously reflected in their fetuses. Other possibilities considered in attempts to explain the fetal respiratory acidosis included the occasional uterine contractions commonly observed in such acute preparations, as well as the fact that we simply may have been witnessing and documenting deteriorating preparations. Since the total oxygen content of the fetal lambs was observed to be decreasing during the study, a portion of the decrease in pH may have been due to an uncompensated metabolic acidosis caused by anaerobic metabolism by the fetus and accumulation of lactate.

In summary, under the conditions of our study, the efficacy of Fluosol-DA (20%) as a volume expander during an isovolemic exchange transfusion in the fetal lamb in utero was demonstrated, since fetal blood pressure was maintained despite an almost 50% acute drop in fetal hematocrit. In the presence of maternal hyperoxygenation, fetal lambs so exchanged were able to maintain a physiologic  $PO_2$ , although the total fetal oxygen content decreased as the hematocrit dropped. Because of the oxygen-carrying properties of the perfluorochemical emulsion, the fraction of fetal oxygen carried in the dissolved state increased significantly compared to saline solution-exchanged controls.

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## Oxygen consumption in fetal lambs after maternal administration of sodium pentobarbital

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To determine whether anesthesia lowers fetal oxygen consumption, sodium pentobarbital (10 mg/kg) was given intravenously to seven chronically instrumented pregnant ewes (123 to 144 days' gestation). Oxygen consumption fell by 23% in association with a rise in fetal vascular Po<sub>2</sub>. Fetal breathing movements were abolished for 50.5 minutes, while the number of fetal heart rate accelerations fell by 80% in the first 30 minutes after pentobarbital injection. It is concluded that anesthesia reduces fetal oxygen consumption, probably by abolishing skeletal muscle activity, and perhaps also by reducing cerebral metabolic rate. (*AM J OBSTET GYNECOL* 1986;154:674-8.)

**Key words:** Fetal lamb, oxygen consumption, anesthesia

Although there have been many reports on various effects of anesthetic agents in the fetal and neonatal periods,<sup>1</sup> there is a lack of information on the effects of general anesthetic agents on fetal oxygen uptake. Since such agents generally readily cross the placenta, they cause anesthesia in the fetus. This might be expected to reduce fetal oxygen consumption by abolishing fetal body movements and breathing activity,<sup>2,3</sup> and perhaps by reducing cerebral metabolism.<sup>4</sup> Alternatively fetal oxygen transfer could be reduced by per-

turbations in uterine blood flow.<sup>1</sup> To address this question, oxygen consumption, blood gas tensions, and pH were measured in chronically catheterized lambs before and after the intravenous administration of sodium pentobarbital to the ewe.

### Methods

**Preparation of animals.** Experiments were performed on seven pregnant sheep. At 116 to 131 days' gestation, surgery was performed under halothane anesthesia, following induction with Pentothal. The uterus was exposed through a midline abdominal incision, and after opening the uterus, silicone rubber catheters were placed in a fetal femoral artery, lateral tarsal vein, the common umbilical vein, trachea, and amniotic cavity. In one fetus an electromagnetic blood flow transducer (C & C Instruments, Culver City, California) was placed on the common umbilical artery.<sup>5</sup>

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Catheters were also placed in a maternal femoral artery and jugular vein. All fetal catheters and leads were tunneled subcutaneously to the ewe's flank and stored there in a pouch.

After surgery the ewes were kept in the company of other sheep in pens. Ampicillin (500 mg) was given intramuscularly to the ewe for the first 3 days after surgery and to the amniotic cavity daily for the duration of the preparation.

**Experimental protocol.** Experiments were performed on the sheep 4 to 24 (mean = 9) days after surgery, at a gestational age ranging from 123 to 144 (mean = 132) days. On the morning of an experimental day a ewe was transferred from the holding pen to an adjacent monitoring cart. After a 90-minute control data-collection period, sodium pentobarbital (Somnotol, MTC Pharmaceuticals, Hamilton, Ontario) was administered intravenously to the ewe at a dose of 10 mg/kg. Five minutes before and 10, 30, and 60 minutes after the injection, paired, 2.0 ml blood samples were collected from the fetal femoral and umbilical venous catheters for the measurement of blood gases, pH, oxygen content, and antipyrine concentration. The blood taken was replaced with an equal volume of maternal blood. Two hours after pentobarbital administration, the ewe was returned to the holding pen.

**Physiological measurements.** During the whole of the experimental period, fetal arterial, tracheal, and amniotic fluid pressures were measured with strain-gauge manometers (Statham model P23Db, Gould Inc., Oxnard, California). Fetal heart rate was measured from the arterial pulse pressure by means of a cardiometer (Model 9857, Sismomedics, Anaheim, California). In the one fetus in which an electromagnetic flowmeter was implanted, umbilical blood flow was measured with a Statham SP2202 blood flow transducer (Gould Inc.). All of these variables were recorded on a polygraph recorder (Beckman R-612, Sismomedics) at a paper speed of 6 or 15 cm/min. Fetal arterial pressure was corrected for amniotic fluid pressure in subsequent analyses. Episodes of rapid, irregular breathing movements and large-amplitude "gasps" or "sighs" were identified from the recordings of tracheal pressure.

Umbilical blood flow in six of the seven experiments was measured by the steady-state antipyrine diffusion technique.<sup>3,6</sup> For this purpose antipyrine was infused continuously via the tarsal vein catheter at a rate of 9.6 mg/min, leading to a fetal arterial plasma antipyrine concentration averaging  $8.0 \pm 0.3$  mg/100 ml. Antipyrine concentrations were measured as described previously.<sup>3</sup> Fetal blood  $PO_2$ ,  $PCO_2$ , and pH were measured with an IL Micro 13 blood gas analyzer (Instrumentation Laboratories, Lexington, Massachusetts) set at 39° C. Blood oxygen content was measured with a

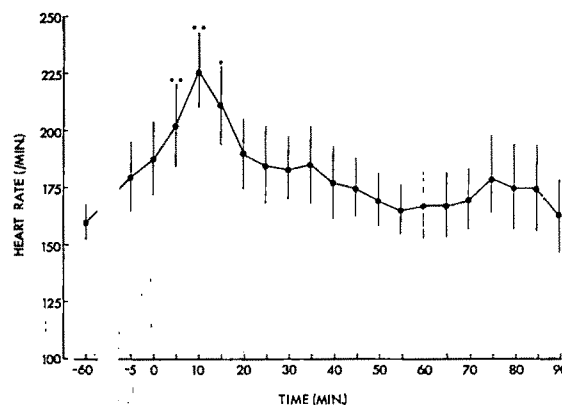


Fig. 1. Mean ( $\pm$ SE) fetal heart rate before and after maternal intravenous administration (at time = 0) of pentobarbital. \* =  $p < 0.05$  and \*\* =  $p < 0.01$  for change from control value.

LexO<sub>2</sub>con-K analyzer (Lexington Instruments, Waltham, Massachusetts).

Fetal total body oxygen consumption was calculated as umbilical blood flow times the umbilical venoarterial difference in blood oxygen content. Fetal weight at the time of experiment was calculated from birth weight as previously described.<sup>3</sup> Results are reported as the mean and standard error of the mean. Changes in the measured variables during the course of the experiment were tested for statistical significance by means of a paired *t* test.

## Results

**Maternal pentobarbital effects.** All ewes were standing when the 10 mg/kg dose of pentobarbital was given. In all but one case the ewes became recumbent within 2 minutes of drug injection and remained anesthetized for  $30.2 \pm 7.7$  minutes (range, 13.5 to 51.0). The remaining ewe remained standing during the experiment and appeared to be only mildly sedated following pentobarbital.

**Fetal breathing and cardiovascular effects.** In the hour before pentobarbital injection fetal rapid irregular breathing movements were present for  $27.8\% \pm 6.7\%$  of the time. In three of the experiments fetal breathing movements were occurring at the time the drug was given, and the movements stopped within 25 to 120 seconds. In all fetuses fetal breathing movements were completely absent in the first 30 minutes after pentobarbital, a significant change ( $p < 0.01$ ) from the control period. Thereafter fetal breathing movements occurred for  $16.8\% \pm 8.5\%$  and  $35.5\% \pm 8.8\%$  of the time in the second and third 30-minute periods after pentobarbital administration, respectively. These values were not significantly different from the value in the control hour. On average, fetal respiratory activity was completely absent for  $50.5 \pm 3.6$  minutes after



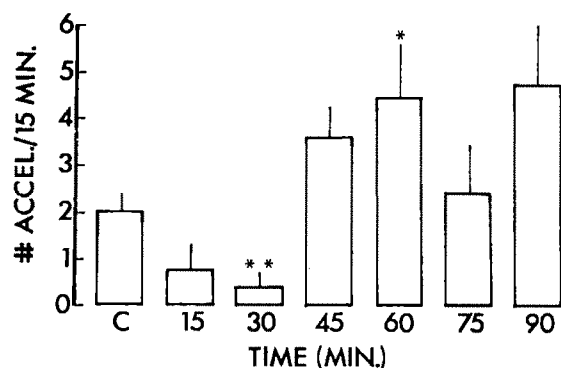


Fig. 2. The number of accelerations in fetal heart rate (as defined in the text) occurring before (C) and over 15-minute intervals after pentobarbital administration. See Fig. 1 for explanation of asterisks.

drug injection. In four of the fetuses the resumption of fetal breathing movements was preceded by the occurrence of slow (once every 1 to 2 minutes), large-amplitude gasps or sighs.

Fig. 1 illustrates the changes in fetal heart rate that occurred during the experiment. In the control hour fetal heart rate averaged  $164.7 \pm 2.7$  bpm. Within 2 to 3 minutes of pentobarbital administration, fetal heart rate began to increase and reached a maximum value of 226.3 at 10 minutes after drug injection. Thereafter fetal heart rate declined gradually, reaching the control level by 50 minutes. The number of accelerations in fetal heart rate (defined as a rise in rate of  $>15$  bpm, lasting at least 10 sec) during the experiment is illustrated in Fig. 2. The value fell following drug injection and was 80% lower than the control value at 30 minutes. Thereafter the number of accelerations rose and became significantly higher than the control value 46 to 60 minutes after pentobarbital administration.

Fetal arterial pressure averaged  $50.6 \pm 0.4$  mm Hg in the control period and was unchanged following pentobarbital injection. There was a tendency for umbilical blood flow (Fig. 3) to increase after drug administration in association with the rise in fetal heart rate; this was most clearly seen in the fetus with the electromagnetic flowmeter. However, the variability in this response was great, and the mean increase at 10 minutes ( $19.7$  ml/min/kg) was not statistically different from zero.

**Fetal oxygen consumption and blood gases.** Fetal oxygen consumption averaged  $6.2 \pm 0.4$  ml/min/kg in the control period (Fig. 3). There was no change by 10 minutes after pentobarbital injection, but by 30 minutes oxygen consumption had fallen significantly by 23% to  $4.8 \pm 0.5$  ml/min/kg. Since umbilical blood flow was unchanged at this point, the fall in oxygen consumption was achieved solely by a decline in the umbilical venoarterial oxygen difference (Fig. 3). At 60 minutes after

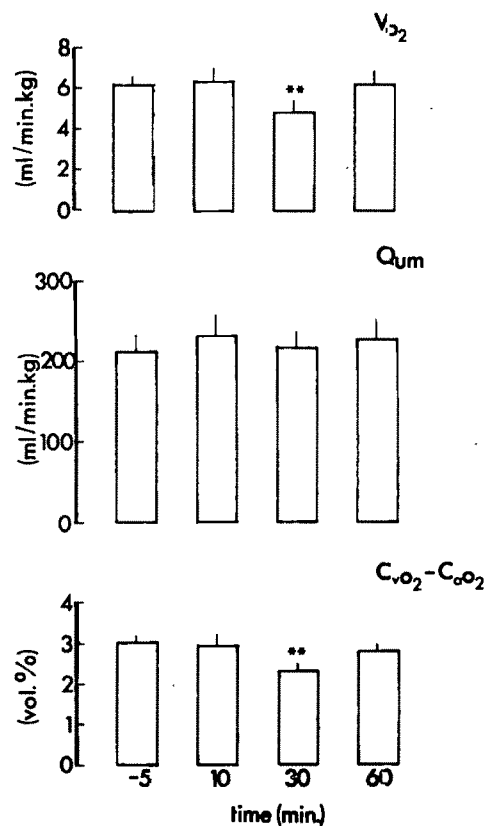


Fig. 3. Fetal oxygen consumption ( $V_{O_2}$ ), umbilical blood flow ( $Q_{um}$ ), and the umbilical venoarterial difference in oxygen content ( $C_{vO_2} - C_{aO_2}$ ) before (-5) and at 10, 30, and 60 minutes after maternal pentobarbital administration. \*\* =  $p < 0.01$  for change from control value.

drug injection, oxygen consumption had returned to the control level.

Mean values for blood gases, pH, and blood oxygen content are given in Fig. 4. Both the umbilical arterial and the venous  $PO_2$  fell slightly but not significantly 10 minutes after drug administration. In association with the fall in oxygen consumption at 30 minutes there was a rise in vascular  $PO_2$ , which achieved statistical significance for the umbilical vein. At 60 minutes  $PO_2$  was not significantly different from the control values.  $PCO_2$  was unchanged during the experiment, while pH declined over the first 30 minutes following pentobarbital administration. pH was largely restored by 60 minutes. Oxygen content values were not significantly changed following drug injection, although there was a slight rise in  $CaO_2$  at 30 minutes.

### Comment

The results of this study clearly show that maternal administration of pentobarbital, at a dose sufficient to cause maternal anesthesia, results in a significant fall in fetal oxygen consumption. It is likely that this effect is in large part due to suppression of fetal activity by

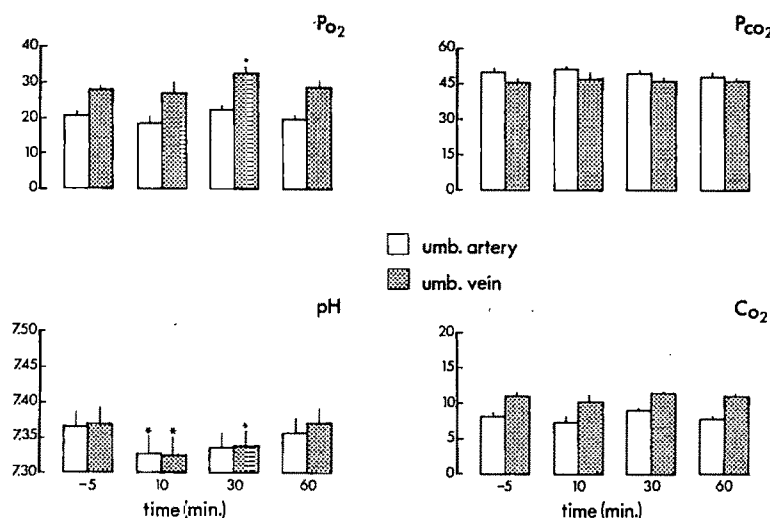


Fig. 4. Fetal umbilical arterial and venous blood gases and pH before (-5) and following pentobarbital administration. \* =  $p < 0.05$  for change from control value.

the barbiturate. Previously we have reported a 30% increase in fetal oxygen consumption during episodes of fetal breathing movements.<sup>3</sup> Conversely, oxygen consumption falls by 15% when the neuromuscular blocking agent gallamine is given to fetuses exhibiting respiratory activity.<sup>2</sup> All barbiturates that have been examined readily cross the ovine placenta.<sup>7</sup> In the present study the fetal breathing movements were arrested for an average of 50.5 minutes after drug injection. Boddy et al.<sup>8</sup> have previously reported that an even lower dose of Nembutal (4 mg/kg of maternal weight) markedly reduces breathing activity in the fetal lamb. Although fetal body movements were not monitored in the present investigation, the reduction in the number of fetal heart rate accelerations in the first 30 minutes following pentobarbital administration is consistent with a decline in body movements, since Bocking et al.<sup>9</sup> have demonstrated that accelerations in fetal heart rate are highly associated with skeletal muscle activity in the fetal lamb. Furthermore, these workers have also demonstrated that, in the majority of cases, this association appears to be due to the simultaneous effect on cardiac sympathetic and motor fibers by central nervous system output. Hence, the decline in accelerations after the drug was administered may be reflective of an attenuation of this central nervous system outflow.

Boddy et al.<sup>8</sup> have reported a gradual change in fetal electrocortical activity from low voltage to high over the first few minutes following administration of 4 mg/kg of pentobarbital to pregnant ewes, and a 50% reduction in the amount of low-voltage electrocorticographic activity in the first hour after drug injection. Since fetal cerebral oxygen consumption is lower during high-voltage electrocorticographic activity than during low-voltage states,<sup>4</sup> it is possible that the effect of pentobarbital

on suppressing low-voltage electrocorticographic activity could contribute to the observed reduction in total-body oxygen consumption.

Mirkin<sup>10</sup> has reported that following injection of 5 mg/kg of pentobarbital to pregnant ewes the fetal plasma concentration of the drug reaches a peak at 20 to 30 minutes and then declines slowly. Therefore it is likely that at 10 minutes after drug injection the levels in the fetus were insufficient for the drug to exert its full effects, explaining the lack of change of fetal oxygen consumption at this time. Although fetal breathing and electrocorticographic activities are altered within a few minutes of maternal pentobarbital administration, it is possible that oxygen consumption in the respiratory muscles and brain would remain normal for longer than this if there were any time lag between the fall in cellular energy requirements resulting from anesthesia and the decline in rate of mitochondrial electron transport and oxidative phosphorylation. Also, during the period of marked fetal tachycardia in the first 15 minutes after pentobarbital administration, it is possible that myocardial oxygen consumption was elevated, thereby masking any decreases in oxygen consumption in other fetal organs.

In the previous study of pentobarbital effects in pregnant sheep, fetal blood gases and pH did not change significantly.<sup>8</sup> In the present investigation, there was a tendency for fetal PO<sub>2</sub> to fall 10 minutes after drug injection. This may have resulted from disturbances in the ewe, such as a decline in uterine blood flow, which has been reported following anesthesia with thiobarbiturates such as thiopental and thiamylal.<sup>1</sup> Transient declines in fetal PO<sub>2</sub> and pH also occur with these agents. The fall in fetal oxygen consumption 30 minutes following pentobarbital administration in the pres-

ent study was accompanied by a significant rise in umbilical venous  $PO_2$  and a slight but insignificant rise in arterial oxygen tension. These changes were probably in part due to the fall in oxygen consumption, since a similar effect occurs after fetal gallamine and pancuronium administration,<sup>2, 11</sup> in which oxygen consumption also falls. But a rise in umbilical venous  $PO_2$  could also be due in part to a reduction in placental oxygen consumption.<sup>12</sup> Such an effect of anesthesia has been postulated by Meschia et al.<sup>13</sup> to explain the lower uteroplacental oxygen consumption measured in anesthetized ewes when compared to conscious, unrestrained animals.

The fetal tachycardia present after pentobarbital administration in the present study is similar to that observed in mature fetal lambs by Boddy et al.<sup>8</sup> The mechanism of this response is unclear. Barbiturates exert depressant effects on sympathetic ganglia,<sup>14</sup> and in pregnant sheep, the maternal and fetal effects of afferent vagal stimulation are abolished during deep pentobarbital anesthesia.<sup>15</sup> The inhibitory ganglionic effects may be responsible for the transient fall in arterial pressure which has been reported following pentobarbital injection in the fetus<sup>8</sup> and in adult nonpregnant dogs.<sup>15</sup> Fetal hypotension was not observed in the present study; the reason for this discrepancy with the published effects of pentobarbital remains to be established.

In summary, pentobarbital anesthesia in the fetal lamb results in a fall in total-body oxygen consumption, probably largely because of a suppression of fetal activity. There are minimal changes in blood gas status, and it appears that short-term use of pentobarbital in pregnancy poses no great risk to the fetus. Indeed, there may be situations, involving a limitation in fetal oxygen supply, in which a pentobarbital-induced reduction in fetal tissue oxygen demands would be of benefit to the fetus by providing some protection from hypoxic damage. A similar argument has been made for protection of the fetal brain by maternally administered barbiturates in cases of fetal asphyxia during labor.<sup>16</sup>

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# Basal and forskolin-stimulated cyclic adenosine monophosphate in intact human platelets during the menstrual cycle

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In previous studies we observed modifications of cyclic adenosine monophosphate and adenylate cyclase activity in human endometrium during the menstrual cycle. In the present study our intention was to verify whether these modifications occur in isolated intact platelets. The results demonstrate that in nine normal women platelet cyclic adenosine monophosphate content varies during the menstrual cycle both in basal and in stimulated conditions (in vitro addition of forskolin). In fact, significantly higher levels of cyclic adenosine monophosphate were consistently observed during the proliferative phase. These findings provide evidence that platelet cyclic adenosine monophosphate metabolism normally varies during the menstrual cycle, which suggests a possible involvement of this system in some important clinical events. (AM J OBSTET GYNECOL 1986;154:679-82.)

**Key words:** Menstrual cycle, platelet, adenylate cyclase, steroid hormones

Evidence that steroid hormones influence cyclic adenosine monophosphate (cAMP) metabolism has been well documented in recent years. In fact, it has been established that the concentration of cAMP is modulated in the endometrium, both in vivo and in vitro, by progesterone and estradiol,<sup>1,2</sup> in monocyte-like cells by 1,25-dihydroxyvitamin D,<sup>3</sup> in the adrenal cortex membranes by cortisol,<sup>4</sup> and in ROS 17/2.8 cells by dexamethasone.<sup>5</sup>

The eventuality that sex steroids influence platelet aggregability, thereby favoring thromboembolic disease, has already been raised with the introduction of steroids as contraceptives.<sup>6</sup> We decided to reinvestigate this phenomenon by measuring intraplatelet cAMP, well known to be an important inhibitor of platelet aggregability.<sup>7,8</sup>

Accordingly, this report presents data that show the influence of the menstrual cycle on basal and forskolin-stimulated cAMP content in intact human platelets.

## Material and methods

**Separation of platelets.** Platelets were isolated from blood samples of nine normal women, ages 18 to 32 years, during the proliferative and secretive phases of the menstrual cycle (between days 6 to 8 and 20 to 24,

respectively). The blood samples were obtained, between 8 and 9 AM, through siliconized venous cannulae to provide 9 ml of blood into 1 ml of ethylenediaminetetra-acetate, 0.1 mol/L (pH 7.4). The entire preparation of the platelet suspension was performed in plastics. Within 15 minutes from collection the platelet-rich plasma was separated by centrifugation at 180 g for 15 minutes in a precooled Sorvall centrifuge (4° C). The pellet was resuspended in 5 ml of ice-cold buffer A (15 mmol/L of Tris hydrochloride, 134 mmol/L of sodium chloride, 5 mmol/L of glucose, and 1 mmol/L of ethylenediaminetetra-acetate, pH 7.4). To remove contaminating red cells, the suspension was centrifuged at 100 g for 9 minutes, and the supernatant (containing primarily platelets) was transferred to a clean tube. To obtain the final platelet suspension this supernatant was centrifuged at 1000 g for 10 minutes and resuspended in 1 ml of ice-cold buffer A.

Aliquots of 0.2 and 0.5 of the platelet suspension were used for determination of cAMP in stimulated and basal conditions, respectively.

**Basal platelet cAMP.** A 0.5 ml aliquot of the platelet suspension in buffer A was centrifuged at 1000 g for 10 minutes (4° C) to separate the platelets. After removing the supernatant, 0.5 ml of ice-cold 6% (wt/vol) trichloroacetic acid was added to the pellet and stirred on a Vortex mixer. A deproteinized extract was obtained by the following steps: centrifugation (1500 g for 10 minutes), neutralization of the supernatant with excess calcium carbonate<sup>1</sup> and recentrifugation at 1500 g for 15 minutes. cAMP was measured in 100 µl samples of the final solution with an antibody-based radioimmunoassay kit, by means of the nonacetylated pro-

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**Table I.** Serum estradiol and progesterone levels with corresponding cAMP content in basal and forskolin-stimulated conditions during the proliferative and secretive phases of the menstrual cycle

Case No.	Patient	Cycle phase*	Blood hormones		cAMP (pmol/10 <sup>9</sup> platelets)		
			Estradiol (pg/ml)	Progesterone (ng/ml)	Basal	Forskolin-stimulated	
						30 sec	300 sec
1	M. A.	P	67	0.2	1.9	56.2	84.7
		S	90	7.4	1.7	8.5	33.3
2	B. C.	P	164	0.6	12.0	33.3	193.4
		S	80	11.0	3.5	19.4	99.6
3	B. B.	P	52	0.1	3.4	39.1	95.8
		S	63	9.9	6.5	42.0	60.0
4	D. F.	P	41	0.2	6.6	70.3	262.0
		S	251	17.0	4.2	41.0	136.9
5	G. L.	P	73	0.3	11.0	28.9	46.3
		S	104	25.0	5.0	18.0	33.2
6	M. L.	P	50	0.3	7.0	67.3	191.8
		S	160	20.0	6.0	41.0	80.0
7	C. C.	P	49	0.3	1.5	14.9	33.6
		S	54	5.4	6.0	32.5	60.8
8	A. B.	P	109	0.3	12.0	64.4	176.6
		S	144	5.7	8.0	26.0	48.2
9	E. L.	P	68	0.1	3.0	30.2	130.2
		S	169	11.4	2.0	12.0	57.6

\*P = Proliferative, S = secretive.

cedure (New England Nuclear, Boston, Massachusetts). The sensitivity of the assay was 0.1 pmol per tube.

**Stimulated platelet cAMP.** Forskolin (purchased from Calbiochem-Behring Corporation, La Jolla, California), isolated from the roots of *Coleus forskolii*,<sup>9</sup> was used to stimulate the platelet cyclase. To quantify the changes of the nucleotide content, we used two aliquots of 0.2 ml of the platelet suspension that were added to the incubation tubes and allowed to equilibrate at 37° C for 300 seconds. Forskolin (in 10 mmol/L of stock solution in ethanol) was then added to the incubation mixture in 50 µl buffer A to a final concentration of 100 µM. Incubation was terminated in the two tubes by addition of 0.5 ml of 6% trichloroacetic acid after 30 and 300 seconds, respectively. cAMP level was then determined by radioimmunoassay in the solutions obtained by deproteinization and neutralization as described above.

**Hormonal analysis.** The serum concentration of estradiol and progesterone were determined by radioimmunoassay with the use of kits by Radioisotope Service (Wurenlingen, Switzerland) and by Diagnostic Products Corporation (Los Angeles, California), respectively. The sensitivities of the assays were 1 pg and 10 pg per tube; intraassay and interassay variations in any case were <10% and <12%, respectively.

**Statistical analysis.** Paired Wilcoxon's test was used for the statistical evaluations.

## Results

The individual results of the measurement of platelet cAMP during the menstrual cycle (expressed in

pmol/10<sup>9</sup> platelets) are listed in Table I. The mean (±SEM) basal values were 6.5 ± 0.15 and 4.8 ± 2.1 in the proliferative and secretive phases, respectively. Thus in the secretive phase significantly lower cAMP levels are present inside platelets. Upon forskolin stimulation the corresponding cAMP values were 44.9 ± 6.7 and 26.7 ± 4.3 at 30 seconds, and 134.9 ± 25.0 and 67.7 ± 11.1 at 300 seconds after the addition of the alkaloid. The resulting differences between values in the proliferative and secretive phases, in all conditions, proved statistically significant at the 0.05 level. As shown in Figs. 1 and 2, upon forskolin stimulation, at 30 seconds intracellular cAMP was raised 9.4 ± 2.7 times over the basal level during the proliferative phase and 4.8 ± 0.6 times during the secretive phase. At 300 seconds, the corresponding increments were 25.0 ± 4.4 times and 12.4 ± 3.2 times. According to these data, "secretive platelets" display a lower level of cAMP and a lower sensitivity to forskolin than do "proliferative platelets." In search of a link between the endogenous hormonal pattern and the observed platelet cAMP values and the response to forskolin, a correlation analysis was performed between the cAMP concentration and the corresponding concentration of circulating hormones, but no significant relationship was found (data not presented).

## Comment

It has previously been established that several platelet functions are subjected to regulation by cAMP.<sup>7</sup> Among these, aggregation was found to be inhibited by substances that increase the intraplatelet level of the nu-

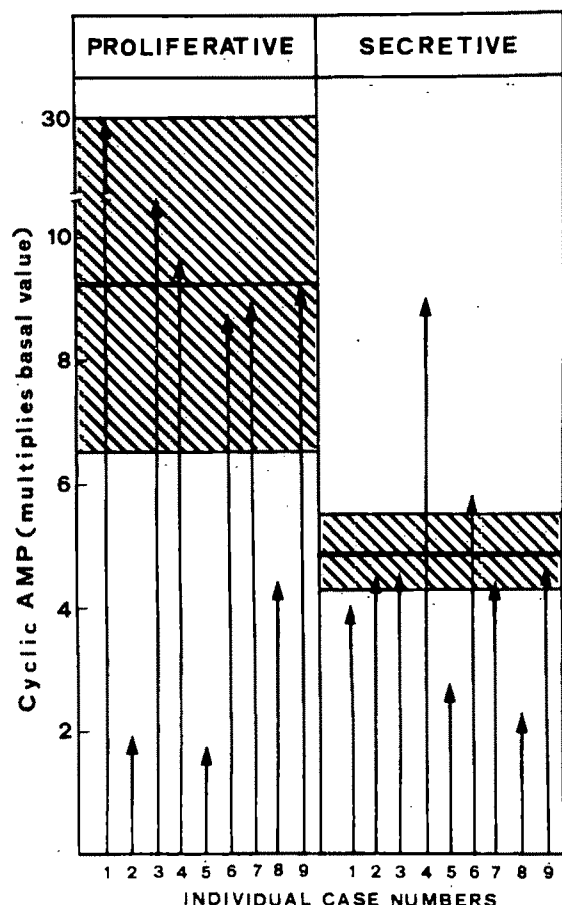


Fig. 1. Elevation of platelet cAMP after 30 seconds of forskolin stimulation. The arrows represent the individual increments in the nine cases studied, during the proliferative and secretive phases, respectively. The solid bars and shaded areas represent the mean increase and SEM, respectively. Individual case numbers correspond to those represented in Table I.

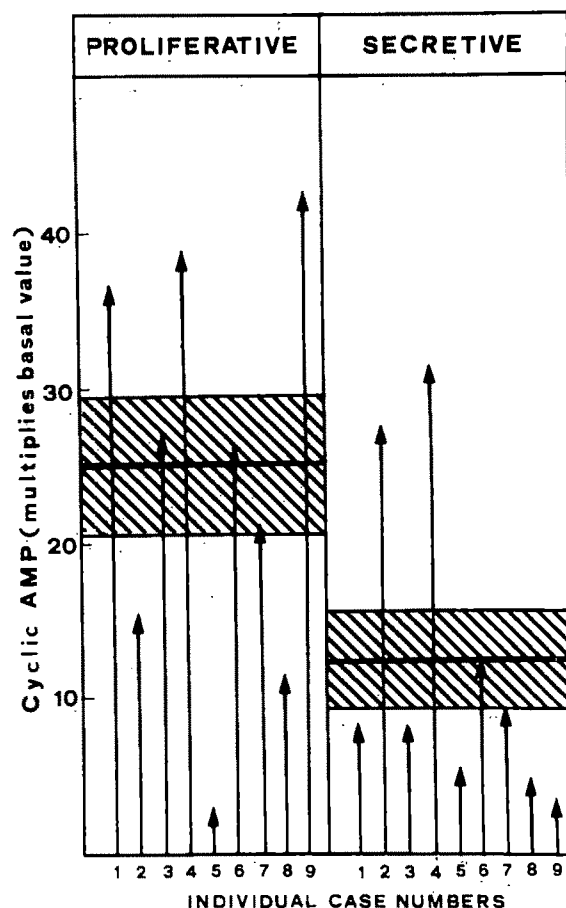


Fig. 2. Elevation of platelet cAMP after 300 seconds of forskolin stimulation. The arrows represent the individual increments in the nine cases studied, during the proliferative and secretive phases, respectively. Individual case numbers correspond to those represented in Table I.

cleotide (for example, prostaglandins, forskolin).<sup>8</sup> In light of these findings we decided to determine the influence of endogenous sex steroids on cAMP levels in the platelets, since previous studies have demonstrated that, at least in some systems, steroid hormones can modulate cAMP metabolism.<sup>1-5</sup> We observed significant changes in platelet cAMP during the menstrual cycle in normal women, with a 30% lower level of cAMP during the luteal phase. In addition to this finding, "cyclic" results were obtained with the forskolin-stimulated platelets, and as before, a lower increase of cAMP was observed during the luteal phase. Since the alkaloid is thought to directly activate the catalytic subunit of adenylate cyclase independently of hormonal receptors and regulatory subunits,<sup>9</sup> the cAMP generation assay suggests the presence of lower levels of the catalytic subunit in this platelet population.

It is noteworthy that our results are in contrast to those reported by Rosen et al.<sup>10</sup> These authors, during studies on  $\alpha_2$  and  $\beta_2$  receptors in mononuclear lym-

phocytes and platelets, examined the influence of circulating sex steroids on adenylate cyclase activity in basal, sodium fluoride-stimulated and epinephrine-inhibited conditions. Their conclusion was that the sex steroids had no influence. This discrepancy may possibly depend on the use of two different experimental systems (isolated membranes versus intact platelets) or on the measured response that in our case is a function of the catalytic subunit and in their study is a function of the regulatory subunit.

In any case, the reason for the change we observed is unclear, since no direct correlation could be established between circulating hormones and physiologic response. Two mechanisms can be hypothesized to account for the lower steady-state levels of catalytic subunits encountered during the luteal phase: either some catabolic mechanism is triggered by the increased levels of progesterone in the blood stream or a diminished synthesis of the catalytic subunit takes place in the platelets in the follicular phase, which represent prev-

alently the circulating platelets in the midsecretive phase, owing to the reported half-life of platelets.<sup>11</sup>

From a physiologic point of view it must be underlined that no clear in vitro modification of platelet function has been detected along the menstrual cycle<sup>6,12</sup>; it is nevertheless conceivable that a change in the circulating sex steroid pattern can affect the ability of the platelets to accumulate the endogenous antiaggregant cAMP, thus leading to an increased susceptibility to circulatory accidents. The likelihood of these concepts must be verified by monitoring cAMP levels and platelet functions, for instance, in oral contraceptive users and in ovarian dysfunction syndromes, eventually with uterine bleeding.

We acknowledge the excellent technical assistance of Ms. Patrizia Cariani and Ms. Stefania Ferrazzini of the Laboratory of Reproductive Biochemistry and Endocrinology, Department of Obstetrics and Gynecology, University of Ferrara. We are also grateful to Dr. G. C. Candini (Health Physics Service, Sanitary Local Unit No. 31, Ferrara) for his contribution in statistical analysis.

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# Extracorporeal perfusion of the human uterus

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Uterine specimens specially prepared for extracorporeal perfusions (arterial and venous stumps available for catheterization) were perfused with oxygenated Krebs-Ringer bicarbonate-glucose buffer for periods of up to 12 hours to investigate the feasibility of obtaining constant flow, stability of biochemical parameters, and adequate distribution of the perfusion fluid. Flow rates of 10 to 30 ml/min per artery could be maintained at pressures ranging from 80 to 120 mm Hg. Arteriovenous gradients of oxygen and carbon dioxide tensions were relatively stable and levels of lactate, lactic dehydrogenase, and creatine kinase released to the medium, indicators of tissue hypoxia or cell lysis, declined after 30 minutes of perfusion, remaining low and stable up to 12 hours. Distribution of methylene blue and radiopaque solutes was practically complete throughout the fundus and upper two thirds of the uterus. A mixture of tritium-labeled estrone sulfate and carbon 14-labeled estrone was injected as a bolus through an arterial catheter during perfusion. Perfusate samples were collected for 30 minutes, and tissue samples were taken at the end of this period. Tritium/carbon 14 ratios in myometrium and perfusate indicated preferential uptake of the unconjugated estrogen. Tritium/carbon 14 ratios were higher in endometrium than in myometrium, which suggests an enhanced permeability of endometrial capillaries to estrone sulfate. (AM J OBSTET GYNECOL 1986;154:683-8.)

**Key words:** Extracorporeal uterine perfusion, permeability, estrone, estrone sulfate

Extracorporeal perfusion of the human uterus offers a new approach for physiologic and biochemical studies of the myometrium, the endometrium, and the uterine vasculature. It can be expected that, as demonstrated in other organs,<sup>1,2</sup> uterine perfusions with oxygenated media through uterine arteries could maintain the tissue viable and responsive to hormones for periods of time sufficient to carry out specific observations and experiments.

The purpose of the present study was to evaluate (1) the feasibility of preparing surgical specimens suitable for catheterization and perfusion, (2) the distribution of the perfusion medium throughout the myometrium and the endometrium, (3) the stability of perfusion rates and biochemical parameters indicative of tissue vitality, and (4) the relative uptake of estrone and estrone sulfate by uterine tissue.

## Material and methods

**Surgical specimens.** The nine uteri studied were obtained from patients undergoing hysterectomy for cervical carcinoma, leiomyomas, or prolapse at the S.

Orsola General Hospital, University of Bologna. The surgical specimens, with stumps suitable for cannulation of the left and right uterine arteries and their corresponding veins, were obtained by standard surgical procedures, avoiding traction and laceration.

**Perfusion procedures.** Immediately after removal of the uterus, 50 ml of warm Krebs-Ringer bicarbonate-1% glucose buffer at pH 7.4 with 2500 U of heparin was flashed through each uterine artery. Epidural cannula, size 16 G (Lab Portex, S.A.Z.I. de la Vigogne, Beck-sur-Mer, France), about 8 cm long, and Abbocath-T, size 16 G (Abbott Ireland Ltd, Sligo, Ireland), about 5 cm long, were used for arterial venous catheterization, respectively.

The perfusion medium was forced by separate roller pumps through silicone rubber tubing coiled inside an oxygenation flask filled with a circulating 95% oxygen/5% carbon dioxide gas mixture and connected to the arterial catheters where pressure was monitored. Samples of perfusion medium and perfusate were taken from arterial and venous catheters with small syringes to determine pH and oxygen concentrations, and perfusate fractions were collected for output flow measurements and biochemical analyses. The complete perfusion system described in Fig. 1 was used to obtain most of the reported biochemical data.

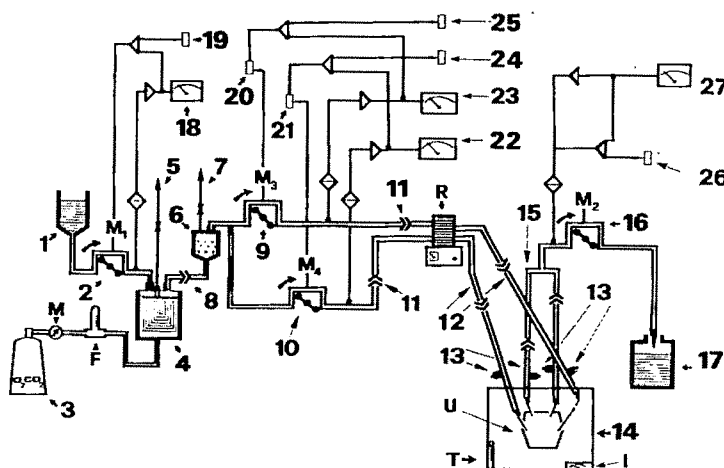
At the end of the perfusion, various samples of myometrium and endometrium were taken with a Tru-Cut biopsy needle (Travenol Laboratories, Deerfield, Illinois) or a Novak curette, respectively. These samples

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**Fig 1.** Diagram of the perfusion system (Refar Mirandola, Italy): 1, reservoir for perfusion buffer; 2, 9, 10, 16, roller pumps; 3, 95% oxygen/5% carbon dioxide tank with standard gage [M] and flowmeter [F]; 4, oxygenation chamber containing about 5 m of silicone rubber tubing (Dow Corning Corp., Midland, Michigan, "medical grade," 0.58 inches in inside diameter, 0.77 inches in outside diameter); 5, gas vent; 6, bubble trap with breather pipe 7; 8, optional heater;  $M_1$ ,  $M_2$ ,  $M_3$ , pressure transducers which are connected to voltmeters, 18, 22, 23, 27, and potentiometers, 19, 20, 21, 24, 25, 26, capable of controlling flow rates (pressure values depend on tubing, catheter and vascular resistances; vascular resistances are estimated by subtracting tubing and catheter contributions from total pressure values); R, thermostatic heating element; 11, 12, arterial catheters with sampling ports 13; 14, temperature and humidity controlled perfusion chamber with hygrometer, 1, and thermometer, T; 15, venous catheters with sampling ports, 13; 17, reservoir to collect perfusate (drained fluid can be totally or partially recycled through reservoir, 1.)

were used for light and electron microscopy or for determination of tritium and carbon 14 levels during perfusions with labeled estrogens.

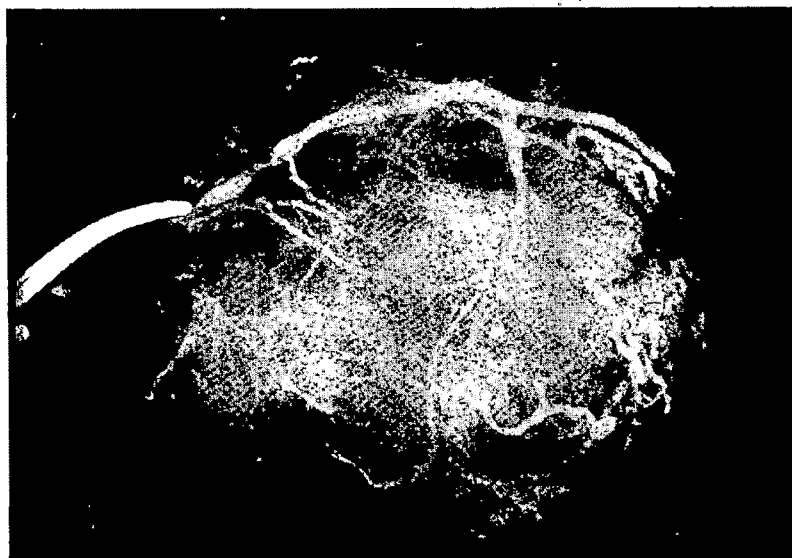
**Injection of mixtures of tritium-labeled estrone sulfate and carbon 14-labeled estrone.** [6,7-Tritium]-labeled estrone sulfate, ammonium salt (specific activity, 52.5 Ci/mmol), and [4- $^{14}$ C]-labeled estrone (specific activity, 57 mCi/mmol) were obtained from New England Nuclear Corp., Boston, Massachusetts. Tritium-labeled estrone sulfate was purified before use by thin-layer chromatography on silica gel 60 F (Merck, Darmstadt, West Germany) twice, with use of ethyl acetate/methanol/concentrated ammonia (75:25:4) and benzene/methanol/acetone (5:1:1) as solvent systems.  $^{14}$ C-labeled estrone was purified on a Sephadex LH-20 (Pharmacia, Uppsala, Sweden) column eluting with benzene/methanol (85:15) and by thin-layer chromatography on silica gel 60 F<sub>254</sub> with use of benzene/methanol/acetone (5:1:1) as the solvent system. A mixture of these two tracers was dissolved in ethanol to obtain concentrations of approximately  $34 \times 10^7$  dpm of tritium-labeled estrone sulfate and  $12 \times 10^7$  dpm of  $^{14}$ C-labeled estrone per milliliter; 0.1 ml of this solution was injected as a bolus into one of the arterial catheters with use of a 100  $\mu$ l Hamilton syringe (Hamilton Bonaduz A.G., Bonaduz, Switzerland) about 1 hour after the perfusion had been established. Teflon tubing was used on the venous side whenever possible to avoid losses of

tracers by adsorption of labeled steroids to polyvinyl, polyethylene, or silicone rubber surfaces<sup>3</sup> during the perfusions. Perfusate fractions were collected every minute for 30 minutes and concentrations of tritium and  $^{14}$ C levels in the perfusate were determined by mixing 0.2 ml of the sample with 1 ml of Soluene (Packard Instruments, Chicago, Illinois) and 15 ml of Dimilume 30 (Packard Instruments) and measuring radioactivity levels in an LKB 1215 Rackbeta II scintillation spectrometer (LKB-Produkter AB, Bromma, Sweden). Tritium and  $^{14}$ C levels in a single sample (approximately 200 mg) of endometrium or in six to eight samples of myometrium obtained from each uterus were determined after dissolving the tissue in 1 ml of Soluene and 15 ml of scintillation fluid.

**Sample analyses.** Oxygen and carbon dioxide partial pressures ( $PO_2$ ,  $PCO_2$ ) and pH in samples taken from the arterial and venous catheters were measured with a BM S3, MK2 Blood Micro System Radiometer, Copenhagen, Denmark. Lactate, lactic dehydrogenase, and creatine kinase were also measured in arterial and venous samples with use of kits obtained from Boehringer, Mannheim, West Germany, which are based on standard methods.<sup>4-6</sup>

## Results

Perfusions were considered to be adequate when constant flow rates (10 to 30 ml/min through each artery)



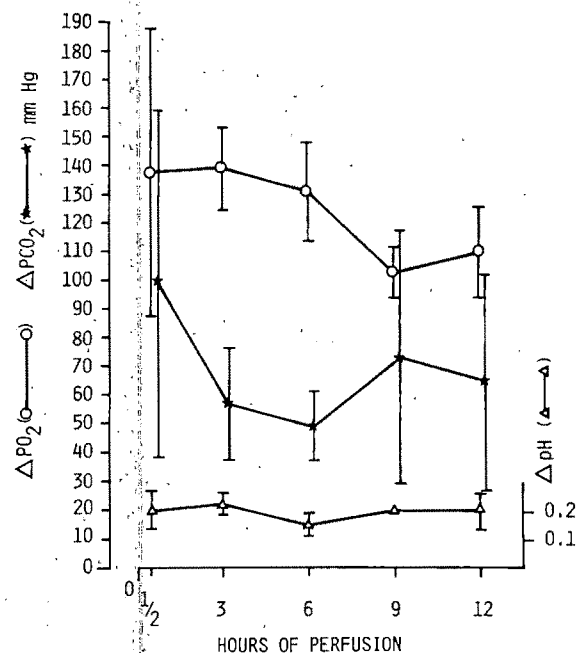
**Fig. 2.** X-ray study shows the distribution of contrast medium into a uterine myoma, under the experimental conditions used for perfusion of the organ. The uterus is well supplied with arcuate and radial arteries crossing vertically and horizontally to form a network of small anastomoses which results in perfusion of the entire organ.

could be maintained at pressures ranging from 80 to 120 mm Hg. Each of the withdrawal pumps connected to the venous catheters was automatically regulated (Fig. 1). Negative pressures of 10 to 15 mm Hg at the venous catheters facilitated the collection of adequate samples of perfusate for biochemical evaluations. Preliminary tests with use of methylene blue indicated full distribution of the perfusion medium throughout the fundus and two thirds of the upper portion of the uterus, including the endometrium. Fig. 2 shows the distribution throughout the myometrium of x-ray opaque material (Isopaque 440, Nyegaard and Co., A/S, Oslo, Norway) injected through both arteries.

Arterial-venous gradients of  $PO_2$ ,  $PCO_2$ , and pH at different perfusion times (0.5 to 12 hours) are shown in Fig. 3. It can be seen that oxygen consumption remains stable during 6 hours of perfusion or longer and that the pH of the perfused buffer does not change significantly.

Fig. 4 shows the levels of lactate as well as lactic dehydrogenase and creatine kinase activities as a function of perfusion time. Hypoxia leading to formation of lactic acid, still noticed during the first 0.5 hours of perfusion, was corrected as the perfusion with oxygenated buffer proceeded. Similarly, levels of lactic dehydrogenase and creatine kinase activity released by the tissue indicated that cytolytic processes occurring during the preparation for perfusion subsides with time. These indicators show stability of the uterine preparation during a 12-hour perfusion period.

Examination by light and electron microscopy showed good preservation of endometrial intracellular



**Fig. 3.** Arteriovenous gradients of oxygen and carbon dioxide partial pressures ( $\Delta PO_2$ ,  $\Delta PCO_2$ ) and of pH ( $\Delta pH$ ) at different times during five uterine perfusions (mean  $\pm$  SD). The graph indicates that oxygen consumption by the uterus is maintained throughout the perfusion period and that the pH of the buffer remains constant.

structures and absence of intracellular edema after 12 hours of perfusion (Fig. 5).

Preliminary results on uptake of unconjugated and sulfated estrogens were obtained with four uteri from

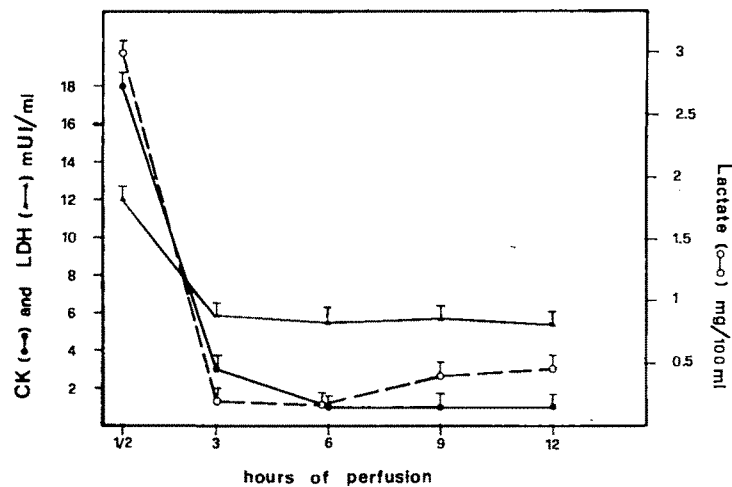


Fig. 4. Lactate concentrations and lactic dehydrogenase (LDH) and creatine kinase (CK) activity levels in perfusates (mean  $\pm$  SD) at different times of perfusions. The graph shows decline of levels of these markers of hypoxia and cytolytic processes after 0.5 hours of perfusion and stabilization for several hours.

Table I. Relative uptake of tritium-labeled estrone sulfate and  $^{14}\text{C}$ -labeled estrone administered as a bolus during uterine perfusions with Krebs-Ringer bicarbonate-glucose

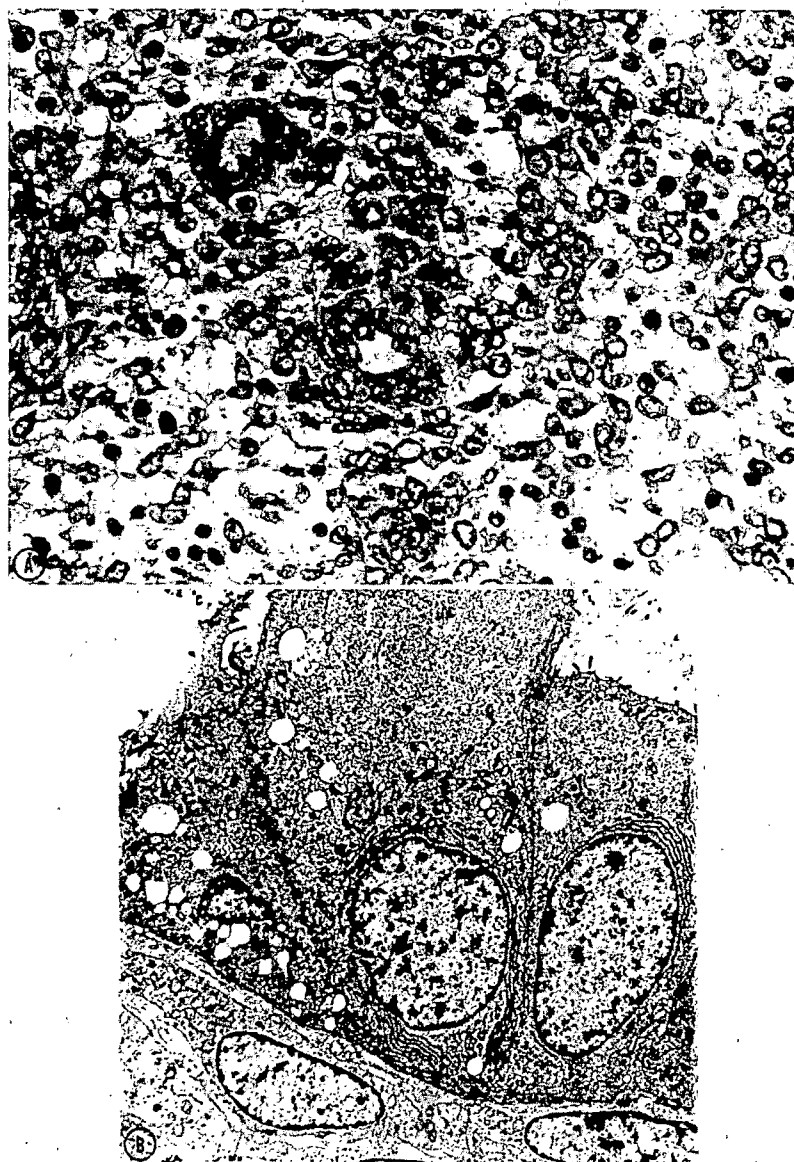
	Experiment				Average
	1	2	3	4	
Tritium/ $^{14}\text{C}$ ratio in the bolus					
dpm of tritium-labeled estrone sulfate/dpm of $^{14}\text{C}$ -labeled estrone	2.3	1.7	2.5	2.8	
Normalized	1.0	1.0	1.0	1.0	1.0
Normalized cumulative tritium/ $^{14}\text{C}$ ratio in perfusate at (min)					
1	4.8	11	1.8	3.2	5.2
2	5.2	12	1.6	3.2	5.5
3	6.1	12	1.8	2.5	5.6
4	5.7	11	1.9	2.6	5.3
5	5.2	11	2.0	2.6	5.2
30	3.4	10	3.0	3.1	4.9
Normalized tritium/ $^{14}\text{C}$ ratio in tissue at 30 min					
Endometrium	3.7	4.1	9.3	3.7	5.2
Myometrium (mean $\pm$ SD)	0.77 $\pm$ 0.15 n = 8	0.80 $\pm$ 0.22 n = 6	0.74 $\pm$ 0.19 n = 7	0.62 $\pm$ 0.17 n = 8	0.73

patients with cervical cancer. Mixtures of tritium-labeled estrone sulfate and  $^{14}\text{C}$ -labeled estrone were administered as a bolus during perfusions with Krebs-Ringer bicarbonate-glucose buffer, and measurements of tritium/ $^{14}\text{C}$  ratios in the perfusate and tissue indicated an overall preferential uptake of  $^{14}\text{C}$ -labeled estrone. As shown in Table I, the tritium/ $^{14}\text{C}$  ratios in the perfusate were found to be higher than the isotope ratios in the administered tracer mixture. This difference was noted in samples of perfusate collected during the first minute following the injection of the tracers, and at 5 minutes the cumulative tritium/ $^{14}\text{C}$  ratio in the collected perfusate was about fivefold higher than the

isotope ratio in the injected bolus. The low tritium/ $^{14}\text{C}$  ratios found in samples of the myometrium indicate a restricted uptake of tritium-labeled estrone sulfate from the perfusion medium. In contrast, the average isotope ratios in the endometrium were similar to those in the perfusates and markedly higher than those in the radioactive bolus (Table I). The endometrium of each of the four patients studied was histologically dated as secretory.

#### Comment

This study demonstrated the feasibility of conducting perfusions of human uteri at  $37^\circ\text{C}$  for periods of



**Fig. 5.** A, Endometrial histologic study of a uterine myoma perfused for 24 hours with Krebs-Ringer bicarbonate-glucose buffer ( $\times 330$ ), showing a good preservation of the endometrial cells supplied by two microarteries (luteal phase). B, Electron micrograph ( $\times 4500$ ) showing good preservation of endometrial cells in another uterine myoma (luteal phase) perfused for 12 hours.

up to 12 hours. Specimens suitable for perfusion were obtained without difficulties in most attempts. Perfusions with oxygenated Krebs-Ringer bicarbonate-glucose buffer could be maintained at  $37^{\circ}\text{C}$  for several hours at a steady rate of oxygen consumption and low output of lactate or intracellular enzymes that serve as markers for cytolytic processes, with preservation of histologic integrity.

We used labeled estrogens to illustrate one application of the uterine perfusion system, namely, to obtain information on the relative uptake of unconjugated and sulfated estrogens. Interest in the utilization of estrone sulfate, the estrogen present in highest con-

centration in blood of nonpregnant women,<sup>7</sup> was prompted by the finding that estrone sulfate could be very efficiently converted to estrone and estradiol during incubations with human endometrium.<sup>8</sup> The question was raised, however, whether circulating estrone sulfate is available to endometrial cells *in vivo*, since tight binding to plasma proteins and barriers to the movement of the negatively charged conjugated estrogen across capillaries could prevent its access to intracellular spaces. Recent *in vivo* studies in rabbits and rats have demonstrated that uterine transcapillary passage of estrone sulfate is much lower than that of estrone.<sup>9, 10</sup> The results reported here indicate that the



fraction of estrone leaving the uterine vascular bed is considerably higher than that of estrone sulfate. The lower uptake of tritium-labeled estrone sulfate was also reflected in the reduced tritium/ $^{14}\text{C}$  ratios found in the myometrium. Interestingly, the isotope ratios in the endometrium were considerably higher than those in myometrium and similar to ratios in the perfusate; this observation may indicate a different capillary permeability in myometrium and endometrium. Trapping of perfusion medium in obliterated endometrial vessels may result in an artifactual increase in tritium-labeled estrone sulfate diffusion into the endometrial tissue but would not account for the observed tritium/ $^{14}\text{C}$  ratio, which was greater than the isotopic ratio in the injected bolus. Estimation of the contribution of circulating estrone sulfate to the intracellular estrogen pool of uterine target cells is a worthy objective that could be pursued with these uterine preparations.

Actions of hormones on enzymatic activities, receptor levels, and output of secretory products in the human endometrium and myometrium, difficult to study in vivo, are currently being investigated in several laboratories by incubating tissue fragments or monolayer cultures of endometrial and myometrial cells in the presence or absence of hormones. Similar metabolic and endocrine studies could be conducted during uterine perfusions maintaining the organ architecture and intercellular relations under optimal conditions for oxygenation, nutrition, and dispensation of exogenous test compounds at no risk to the patient. Furthermore, a variety of techniques are available for the measurement of diffusion, transport, and metabolic parameters during organ perfusions, which could be applied to study the human uterus. Some of these experimental designs are based on perfusions with labeled compounds and measurements of steady-state concentra-

tions of precursors and metabolites in perfusates<sup>11</sup> and others on rapid injection of boluses of a labeled test compound mixed with intravascular, extracellular, or freely diffusible labeled markers, followed by measurement of isotopic ratios in tissue<sup>12</sup> or perfusates.<sup>13</sup>

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## CORRESPONDENCE

### Behavior of the squamocolumnar junction during normal pregnancy

#### To the Editors:

During colposcopic follow-up of patients with abnormal cervical cytologic findings, previous investigators observed outward migration of the squamocolumnar junction during pregnancy.<sup>1,2</sup> We have attempted to assess the applicability of this finding to normal pregnant women based on visual and colposcopic inspections of the cervix.

The investigation involved serial examinations of 15 low-risk gravid women with Class I cervical cytologic studies with use of a Magnavari colposcope. At every visit the squamocolumnar junction was photographed, and colposcopic findings were documented in detail. The patients were black and Hispanic women. Their ages ranged from 18 to 36 years (mean, 24). The mean gravidity was 2.3. The patients were seen at four weekly intervals between the first trimester and term. As an average, each patient had 5.9 colposcopic examinations during the pregnancy.

All patients involved in the study carried their gestation to term. The position of the squamocolumnar junction remained unchanged in 14 of 15 patients throughout the pregnancy (Table I). In seven patients the squamocolumnar junction remained inside, whereas in seven patients it was permanently outside the endocervical canal. In one patient there was evidence of migration of the squamocolumnar junction toward the exocervix while the cervical canal dilated to 1.5 cm by the time of the second visit at 16 weeks. Since



Fig. 1. Patient B-H at 28 weeks' gestation.



Fig. 2. Patient B-H at 32 weeks' gestation.

Table I. Location of squamocolumnar junction

Endocervical	Outside external os
7/14	7/14

spontaneous abortion appeared imminent, this patient was removed from the study and was treated with Schirodkar cerclage. Subsequently the pregnancy continued uneventfully to term.

Our observations appear to indicate that migration of the squamocolumnar junction does not occur in normal pregnant women<sup>1,2</sup> (Figs. 1 and 2). This is contrary to other studies where outward migration of the squamocolumnar junction was observed. The possibility does exist that outward migration occurs as a preliminary event before premature labor. Our single relevant observation of outward migration is consistent with such a possibility. We intend to evaluate this question further by extending our investigation to women at risk for the complications described above.

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### Disagreement on use of water-soluble contrast material in small bowel obstruction

To the Editors:

We read with interest the experience of Watkins and Robertson (*AM J OBSTET GYNECOL* 1985;152:450-5) on the use of water-soluble contrast material in the treatment of postoperative ileus. As is evident from this report, selection of an appropriate contrast agent for evaluation of intestinal obstruction or ileus continues to be a debated issue.<sup>1</sup> However, their contention that Gastrografin has a specific therapeutic action in patients with postoperative ileus remains questionable, as Russell points out in his discussion of the paper. There is no convincing evidence that iodinated contrast agents act differently from other hyperosmolar solutions or that early ambulation and ingestion of liquids, as stated by Russell, would not have the same effect as Gastrografin. Since this study was not controlled to evaluate these factors, the therapeutic role, if any, of water-soluble contrast agents in relieving ileus has yet to be proved.

Water-soluble media may be used for rapid differentiation between mechanical small bowel obstruction and paralytic ileus. An ileus is likely if the contrast material reaches the colon within 1 to 2 hours.<sup>2</sup> However, the average transit time of 3 hours, 20 minutes reported by Watkins and Robertson suggests that some of their patients may have had a partial mechanical obstruction, even if only transiently. The authors did not clearly differentiate ileus from possible obstruction and simply assumed that all their patients had postoperative ileus. Consequently their implication that this technique was invariably effective in diagnosing paralytic ileus is not only misleading but also contradicts other publications showing much poorer reliability.<sup>3,4</sup>

Radiographic examination of the small bowel for evaluation of obstruction is best done with use of barium sulfate suspensions<sup>1,5,6</sup> In particular, enteroclysis has been useful in identifying the level of small bowel obstruction and its potential cause.<sup>7</sup> Because of its poor diagnostic yield, water-soluble contrast material should not be used in assessing intestinal obstruction unless bowel perforation is suspected or the patient requires immediate surgery.<sup>1</sup>

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### Reply

To the Editors:

We appreciate the interest of Ott et al. We do not contend that water soluble contrast is superior in therapeutic effect to other hyperosmolar solutions but rather that water-soluble contrast small bowel study is effective in our experience in relieving distension and pain in postoperative ileus, while effectively excluding perforated viscus and/or high-grade mechanical obstruction, which nonopaque hyperosmolar solutions would not do.

We would agree that this technique may miss low-grade partial or intermittent mechanical obstruction. Any patient with persistent or recurrent symptoms or signs of mechanical obstruction after water-soluble contrast small bowel study should be studied with a barium x-ray examination. However, starting with a barium study, particularly in postoperative patients, eliminates the therapeutic effect in those patients with ileus (the great majority of those studied). Further, barium studies are often prolonged in patients with ileus rather than obstruction, are dangerous in patients with occult perforation, and do not offer ideal bowel content for the surgeon who has to operate on the complete mechanical obstructions thus found.

I first used water-soluble contrast small bowel studies for rapid and safe differentiation of high-grade mechanical obstruction of the small bowel while I was a fellow in abdominal radiology with Dr. Robert Stanley at Mallinckrodt Institute of Radiology in 1970. I found that Dr. Stanley, now at the University of Alabama, continues to use the water-soluble small bowel study for that purpose (personal communication).

I have observed that many radiologists and surgeons have little experience with water-soluble small bowel studies. I have also noted the rapid conversion to enthusiastic acceptance and use of the study by virtually all of the surgeons and radiologists who have practiced

at our hospital in the past 14 years. I also note with interest that two of the four physicians commenting on the paper expressed enthusiasm for their experience with the use of water-soluble contrast small bowel studies in Portland and San Diego.

I agree with Ott et al. that selection of appropriate contrast remains controversial. However, I believe this controversy is important and is ill served by prejudice against a technique that many find of great value and that has not been thoroughly evaluated and reported in the literature.

I would propose that our paper be considered a record of a clinical/radiologic observation that seems to be of value. I would suggest that appropriate controlled studies of barium compared to water-soluble contrast small bowel study in rapid, safe, and accurate differentiation of ileus versus high-grade mechanical obstruction should be carried out. Similar studies of the efficacy of water-soluble contrast small bowel study compared to other hyperosmolar solutions in therapy of postoperative ileus may also be of value. I would enjoy participation in a multiinstitutional study but such studies might be difficult here because of the remarkable enthusiasm for water-soluble contrast small bowel study that exists on the part of the clinical staff in our institution.

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### Correct use of Hegar's sign

To the Editors:

With reference to Dr. Robert A. Munsick's article on Dickinson's sign (*AM J OBSTET GYNECOL* 1985;

152:799), I always thought that the early softening of the uterus was identified as Hegar's sign. At least that is what it is called in all the textbooks on obstetrics. Or am I not keeping pace with modern nomenclature?

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### Reply

To the Editors:

Most modern textbooks correctly describe Hegar's sign, but some incorrectly ascribe and describe other eponymized signs of early pregnancy. C. Reinl, an assistant to Alfred Hegar in Freiburg, Germany, first published a description of what is now known as Hegar's sign. However, Hegar's subsequent publication, greater renown, and the possibility that he taught it to Reinl have combined to leave the eponym totally to Hegar. The most relevant part of Reinl's description is presented here, as translated and reported by Speert<sup>1</sup>:

"This consists in the demonstration of an unusual softness, flexibility, and thinning of the lower uterine segment, that is, of the part directly above the insertion of the uterosacral ligaments.

"This finding is not only demonstrable when the rest of the uterus feels firm, as is often the case, but also very definitely when it is soft and elastic.

"Also in the latter case it is always possible to compress the lower uterine segment, to actually thin it out with the finger, and so to differentiate it from the upper part of the uterus, while it still clearly differs in consistency from the cervix below. The pliability and laxness of these parts can be so extensive that one may be in doubt as to whether any connection exists between the cervix and the larger abdominal or pelvic mass."

Table I. Signs of early pregnancy

Sign and location	Names	Year described	References
Vestibule and vagina			
Violet to blue color of the vaginal vestibule, vagina, or (possibly) cervix	Jacquemin's*	<1836	1, 2, 3
	Kluge's	c. 1835	1, 2, 3
	Chadwick's	1886	1, 2, 3
Violet to blue spot of the vaginal vestibule or anterior vaginal wall, just beneath the urethral meatus, with or without Jacquemin's sign	Chadwick's*	1886	2, 3, 4
Cervix			
Cervical softening	Goodell's*†	1880	5
Uterine isthmus			
Fingertip-sized area of softening at isthmus	Ladin's*	1907	6, 7
Softening of the isthmus	Reinl's	1884	1, 2, 8
	Hegar's	1895	1, 2, 9
Flexibility of the isthmus	McDonald's*	1908	3
Palpable uterine or vaginal arterial pulsations	Pargamine†	?	3
Uterine corpus			
Focal softening, furrows, "bellying," irregularities	Dickinson's*	1892, 1893	See 10 for references
	Piskacek's	1899	
	von Braun-Fernwald's	1899	
Uterine contractions	Braxton Hicks*	1871	11, 12

\*Preferred eponymic name.

†Reference unobtainable to check its validity and date.



In my article on Dickinson's sign, I was referring strictly to focal softening of the uterine corpus and excluded Hegar's and Ladin's signs because they seemed too subjective and variable in degree to allow for definite quantification.

I hope that Table I will diminish some of the confusion concerning the various pelvic signs of pregnancy and that it will identify those eponyms that I believe should be attached to each of these signs.

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#### Relationship between log of the human chorionic gonadotropin concentration and time period in early pregnancy

To the Editors:

I read the paper by Pittaway et al. entitled "Doubling times of human chorionic gonadotropin increase in early viable intrauterine pregnancies" (*AM J OBSTET GYNECOL* 1985;152:299) with great interest, but I am doubtful about the merits of depicting the relationship between the log of the human chorionic gonadotropin (hCG) concentration and the time period in early pregnancy by a polynomial. We know that there is a wide between-patient variation in the hCG doubling time below 6000 to 6500 mIU/ml (~44 days after the last menstrual period),<sup>1</sup> and this cannot be used as *prima facie* evidence of a nonlinear relationship. In our longitudinal study<sup>1</sup> all patients had initial hCG values of <6000 mIU/ml; when we compared the rate of hCG increase with single "low for date" hCG values in the

diagnosis of ectopic pregnancy, no values beyond 44 days after last menstrual period were used to construct the regression line.<sup>2</sup> (The regression line was extrapolated to include a handful of patients, since no fiducial limit, however derived, could have separated the women who had normal and ectopic pregnancies.) It is equally well established that at levels >6500 mIU/ml the rate of hCG increase slows and the between-patient variation widens further.<sup>3,4</sup> This fact coupled with the clear-cut diagnostic capabilities of ultrasound at this stage of gestation<sup>4</sup> removes both the possibility and the need to use serial hCG determinations for patient management (when the values are increasing). Therefore, the central question, at least as far as patient management is concerned, centers on the appropriateness of the exponential model for the hCG-time relationship for hCG values of <6000 mIU/ml, that is, during the first 6 weeks of amenorrhoea. By including cases with hCG values well below this value, the authors have detracted from any answers that their data might otherwise have provided.

However, several other aspects about the design and analysis of Table II, which lies at the heart of this paper, give cause for uncertainty. First, although we are told that the sampling interval was "usually" between 2 to 4 days, it is important to know how many patients had sampling intervals in excess of 4 days and precisely what these intervals were. Second, it is vital to know the variance of the doubling times for the five groups in order to assess the degree of overlap between them. Third, multiple comparisons between several means cannot be made after inspection of the data by a series of *t* tests. Analysis of variance should have been used, and a significant result further evaluated by one of the many available methods (for example, Scheffe's method). Had this been done many of the *p* values quoted would have looked much less impressive and quite probably not "statistically significant." Inasmuch as group 3 duplicated groups 1 and 2 except for four cases, it provides no further information about the central issue at hand, so the point of including it escapes me. (It is noteworthy, however, that this group parallels the range of our own data, except for a few earlier values; the mean doubling time for the group was 1.8 days, whereas in our study it was 1.98 days) We are therefore left with groups 1, 2, and 4 to consider.

Dividing continuous data by inspection into various categories is a hazardous undertaking at the best of times, but the practice is particularly worrisome when the purpose of the analysis is to define the distribution of a continuous variable. Furthermore, if one constrains both the initial and the final hCG values to fall within arbitrary limits, which is what the authors have apparently done, one is unwittingly subjecting the doubling times to an additional selection process. The potential for arriving at spurious results when data are treated in this way must be considered to be very large. What is puzzling, however, is that the mean doubling time for group 4 was greater than that for group 2. Clearly, if a value of, say, 300 ng/ml is constrained to be <1000 ng/ml after at most 4 days (group 2) and the same initial

value is constrained to be greater than 1000 ng/ml (group 4), one would expect the opposite. This paradox suggests that the majority of the patients in this group had initial hCG values close to 5000 mIU/ml and/or long sampling intervals, and to a large extent we are merely seeing the well recognized falloff in the rate of hCG increase again.

In essence, then, the force of the authors' argument rests on the data pertaining to groups 1 and 2. Even if we accept their arbitrary dividing line, it is clearly not feasible from a practical point of view to have separate nomograms of doubling times for these two stages of early gestation, since a significant proportion of patients will defy such a dichotomous classification. In fact, in our experience, well over half of the patients who present with symptoms and have hCG values of <1200 mIU/ml (200 ng/ml) would do so. The issue therefore is not what the distributional form of the rate of hCG increase is (and it may be different in different individuals) but rather how good the linear approximation to it is.

The authors' statement that "a quadratic equation with a negative quadratic term describes the data significantly better than a linear equation" while true (given the nature of their analysis), does not tell the entire story. Figs. 2 and 3 show that the addition of a quadratic term increased the amount of variability "explained" by regression by <4%. In other words, 70.56% of the variation in hCG levels was explained by a linear relationship with "days after last menstrual period," whereas 73.96% of the variation was explained by a quadratic relationship. The corresponding figures for "days after basal body temperature shift" were 77.44 and 79.29, respectively, hardly a striking increase. From a practical point of view, the impact of this gain in precision (which has been inflated by the inclusion of the >6000 mIU/ml group for certain and may even be totally spurious) would be negligible and would certainly not warrant the inclusion of a quadratic term. Nor can it be inferred that its inclusion would increase the diagnostic accuracy of serial hCG measurements, since this is largely determined by the degree of overlap in the rates of hCG increase in normal and abnormal pregnancies. A final point worth making, since it has some methodologic implications, is that although the correlation coefficients "were higher when the gestational age could be estimated more precisely" (that is, from basal body temperature records rather than the last menstrual period), the difference between the correlation coefficients quoted in the paper is not "statistically significant."

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#### Reply

To the Editors:

Dr. Kadar assumed (as did other investigators) that a linear relationship exists between the log of the human chorionic gonadotropin (hCG) concentration and length of gestation before 6 weeks after the last menstrual period (reviewed in Table I of our article). In our report we presented data analyzed with three different methods showing that this is not the case in normal pregnancy. First, sequential linear regression analyses of hCG concentrations revealed a decreasing slope (increasing doubling time) with gestational age before 42 days. Second, the mean doubling time in groups 1 and 2 (<6000 mIU/ml) were statistically different ( $1.6 \pm 0.3$  SD and  $2.0 \pm 0.4$  SD, respectively). Third, a statistical comparison between linear and quadratic equations of the data indicated that the quadratic equation described significantly more of the variability than the linear equation.

Since Kadar objects to the division of continuous data

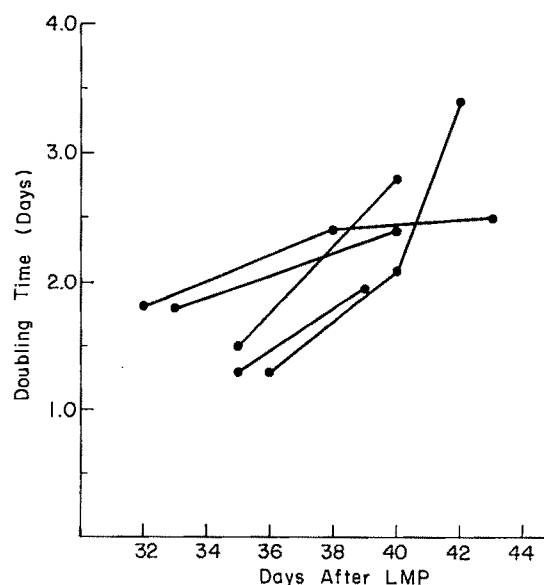


Fig. 1. Doubling times of hCG in five normal intrauterine pregnancies. The doubling time values are shown according to the days of the second hCG determination (after the first day of the last menses).

by inspection (which was not done), a fourth argument for a nonlinear relationship is presented which does not require dividing the data into subgroups. In Fig. 1, the doubling times in five different women who were not in the original study are seen to increase with gestational age before 44 days and do not remain constant as Kadar suggests.

With regard to Kadar's comment that the "impact of this gain in precision (which has been inflated . . . and may even be totally spurious) would be negligible," I would refer him to our study<sup>1</sup> in which we demonstrated that the use of the nonlinear relationship to establish multiple normal values of doubling time was statistically more sensitive in detecting abnormal pregnancies than the use of a single mean doubling time determined by linear regression analysis. In a clinical trial with use of multiple normal values of doubling time and real-time sonography,<sup>2</sup> initial doubling times were abnormal in five of eight asymptomatic women who subsequently were found to have ectopic pregnancies. All eight ectopic pregnancies were identified before tubal rupture, and conservative surgery was performed on six of the eight women.

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#### Reply to Dr. Emanuel Friedman

To the Editors:

In his reply to my letter (AM J OBSTET GYNECOL 1985;153:1006), Dr. Friedman accuses me of *ignoratio elenchi*, the fallacy of irrelevant conclusions. This charge is, of course, groundless, since the main point of my writing was to draw your readers' attention to the many factual inaccuracies and hyperboles contained in original article of Friedman et al. (AM J OBSTET GYNECOL 1984;150:941) lest they became entrenched, as so often happens in medicine. Briefly, these were: (1) that "all controlled studies thus far published" have shown a detrimental effect after midforceps deliveries (which is untrue); (2) that two of the studies cited by Dr. Friedman as controlled studies were in fact retrospective audits without internal controls; (3) that the authors of the single controlled study he cited, which did show an adverse short-term outcome after use of

Kielland's forceps, subsequently examined at 2 years 101 babies delivered with this procedure and found them all to be normal and without handicap; (4) that contrary to Dr. Friedman's claim that those who continue to use midforceps ignore the evidence provided by this study, its findings have been widely discussed, and at least in England, most obstetricians now opt for cesarean section in the presence of fetal acidosis as a direct result of it; (5) that the picture Dr. Friedman paints regarding the use of midforceps is misleading given that evidence both from England and America is available to suggest, surprisingly, that the frequency of midforceps deliveries has in fact increased over the last 20 years or so (these were studies based on reviews done at single institutions, not historical controls, with many cases that Dr. Friedman would classify as midforceps designated as low-forceps or low-midforceps, and the findings stand if one looks only at rotational forceps, which are free from classificatory biases).

It was regrettable that such inaccuracies were allowed to appear in print, and no attempt to disguise them now should be permitted.

The charge of *ignoratio elenchi* also cannot be leveled against my introductory remarks, since these were not made in an attempt to prove or disprove anything but to explain why I thought the study would not have the desired effect of "removing any remaining doubt" from the midforceps controversy. Time may prove me wrong but I doubt it, particularly as I have already had private letters of endorsement from American obstetricians who are not personally known to me.

Finally, I pointed out that Dr. Friedman's analysis lent as much support for abandoning trials of labor as it did for abandoning midforceps deliveries. I did not claim that this ineluctable fact proved or disproved anything (*ipso facto* invalidating the charge of *ignoratio elenchi*); I merely wanted to be certain that your readers had asked themselves the question, "if this study was supposed to remove any remaining doubt about the hazards of forceps, why has it not removed all doubts about the hazards of trials of labor?" There are two reasons why this question must be asked. First, should one accept the interpretation Dr. Friedman prefers, one is duty bound, by the principle of logical and scientific consistency, to abandon trials of labor along with midforceps deliveries. Second, given its design, this study has merely shown that babies who were delivered by midforceps had a slightly lower average intelligence quotient score and that this association was not a likely chance event nor attributable to any linkage between midforceps deliveries and race, parity, or abnormal labor. Since the use of midforceps may well be linked with factors besides race, parity, and abnormal labor, notably, fetal acidosis, the study does not establish the kind of causality Dr. Friedman seeks. If at the same time the evidence relating to another factor which lowered mean intelligence quotient scores to a still greater extent is not believed or at least ignored, then there is all the more reason to be skeptical about the real mean-

ing of these findings, especially when the effects of both these supposedly causative factors could easily be explained by a common linkage with fetal acidosis, the detection of which is known to have been insensitive during the period to which the data relate. I note that in reply to a previous correspondent who raised a similar point, Dr. Friedman (AM J OBSTET GYNECOL 1985;152:604) argued that, "All patients included in the analysis were subjected to essentially elective midforceps delivery. . . . Therefore, any adverse long-term effect on surviving children had to be attributable to the midforceps rather than to the nonexistent indication for midforceps." Nowhere in the Material and methods section of the original paper is it stated that the study was confined to elective deliveries, and consequently there is no comment about any exclusions resulting from intrapartum asphyxia. We do not even have information about the frequency of postmaturity/postmaternity in the various groups, let alone bleeding, bradycardia, etc. In my view such a response to a serious and valid question, given after the fact, is not acceptable.

Dr. Friedman's suppositions about my biases are ill founded, but I will not be drawn into ad hominem argumentation, which is the enemy of honest scientific

debate. However, I would point out that my opposition to accouchement forc  has been recorded.<sup>1</sup> While I await with interest the definitive studies Dr. Friedman has promised us, I am left wondering why it was necessary to anticipate them, what the log logistic model (applicable to binary response variables) to which he repeatedly refers has to do with a study of intelligence quotient scores (a continuous response variable), what the type I error rate is likely to be when 2441 covariates are examined in light of the data, and, perhaps most importantly, what possible relevance the findings could have to contemporary obstetric practice which, as every senior obstetrician I have discussed this with admits, bears little relationship to what was practiced over 25 years ago.

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#### Erratum

In the February, 1986, issue of the JOURNAL, in the article "Heat flux and oxygen consumption of the pregnant uterus," which appears on pages 462-9, the name of co-author Nathan Wasserstrum was inadvertently omitted. The correct listing of the authors should have been: "R. Rudelstorfer, M.D., K. Tabsh, M.D., A. Khoury, M.D., B. Nuwayhid, M.D., C. R. Brinkman III, M.D., N. Wasserstrum, M.D., Ph.D., and N. S. Assali, M.D."



## Books received

**Advances in International Maternal and Child Health.** Volume 4. Edited by D. B. Jelliffe and E. F. Patrice Jelliffe. 153 pages. New York, 1985, Oxford University Press. No price listed.

**Advances in Perinatal Medicine.** Volume 4. Edited by Aubrey Milunsky, Emanuel A. Friedman, and Louis Gluck. 331 pages, illustrated. New York, 1985, Plenum Publishing Corporation. \$45.00.

**Chemically Induced Birth Defects.** James L. Schardein. 904 pages, illustrated. New York, 1985, Marcel Dekker, Inc. \$125.00 (U. S., Canada).

**Childbearing in American Society: 1650-1850.** Catherine M. Scholten. 143 pages. New York, 1985, Columbia University Press. \$22.50.

**Exposure of the Pregnant Patient to Diagnostic Radiations: A Guide to Medical Management.** Louis K. Wagner, Richard G. Lester, and Luis R. Saldana. 187 pages, illustrated. Philadelphia, 1985, J. B. Lippincott Company. \$32.50.

**Gamete Quality and Fertility Regulation.** R. Rolland, M. J. Heineman, S. G. Hillier, and H. Vemer. Amsterdam, 1985, Elsevier Biomedical Press BV. \$74.00 (U. S.).

**Hematopoietic Stem Cell Physiology.** Eugene P. Cronkite, Nicholas Dainiak, Ronald P. McCaffrey, Jiri Palek, and Peter J. Quesenberry. 510 pages, illustrated. New York, 1985, Alan R. Liss, Inc. \$84.00.

**Human Prenatal Diagnosis.** Edited by Karen Filkins and Joseph F. Russo. 424 pages, illustrated. New York, 1985, Marcel Dekker, Inc. \$75.00 (U. S. and Canada).

**Menstrual Disorders and Menopause Biological, Psychological and Cultural Research.** Linda R. Gannon. 285 pages. New York, 1985, Praeger. \$14.95.

**Modern Trends in Infertility and Conception Control.** Edited by Edward E. Walach and Roger D. Kempers. 539 pages, illustrated. Chicago, 1985, Year Book Medical Publishers. No price listed.

**Ovarian Cancer.** Edited by David S. Alberts and Earl A. Surwit. 277 pages, illustrated. Boston, 1985, Martinus Nijhoff Publishers. \$59.50.

**Physiological Development of the Fetus and Newborn.** Edited by C. T. Jones and P. W. Nathanielsz. 837 pages, illustrated. New York, 1985, Academic Press. No price listed.

**Pulmonary Development: Transition from Intrauterine to Extrauterine Life.** Edited by George H. Nelson. 536 pages, illustrated. New York, 1985, Marcel Dekker, Inc. \$75.00 (U. S. and Canada).

**Study Guide and Self-Examination Review for Langman's Medical Embryology.** Fifth Edition. Thomas W. Sadler. 167 pages, illustrated. Baltimore, 1985, Williams & Wilkins. \$14.95 (soft cover).

**Symptoms, Illness and Surgery: A Complete Guide.** H. Winter Griffith. 897 pages. Tucson, Arizona, 1985, The Body Press. \$12.95 (soft cover).

**Touching is Healing.** Dr. Jules Older. 300 pages. New York, 1985, Stein and Day. \$11.95 (soft cover).

Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of \$60.00 U.S. and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to The C. V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146.

**Cesarean Sections: Controversies Over When and Why**, Friday, April 11, 1986 (2:00PM to 8:00 PM, at the Alta Bates Hospital Auditorium, 3001 Colby St., Berkeley, CA 94705. For further information contact: Mary Grim, Medical Education Coordinator. Tel.: (415) 540-1420.

**Postgraduate Course in Medical Ultrasound**, April 14-June 6, 1986. For further information contact: Dr. Frederick W. Kremkau, Director, Center for Medical Ultrasound, Bowman Gray School of Medicine, 300 South Hawthorne Road, Winston-Salem, NC 27103. Tel.: (919) 748-4505.

**Advanced Applied Ultrasound in Obstetrics**, July 31-August 2, 1986, at Snowmass Conference Center, Snowmass, Colorado. For further information contact: Dr. Frederick W. Kremkau, Director, Center for Medical Ultrasound, Bowman Gray School of Medicine, 300 S. Hawthorne Road, Winston-Salem, NC 27103. Tel.: (919) 748-4505.

**Fourth International Forum of Andrology**, Thursday and Friday, June 19 and 20, 1986, at the Hotel Intercontinental, rue de Castiglione, 75001 Paris, France. For further information please contact: Pr. G. Arvis, Department of Andrology-Urology, Hôpital Saint-Antoine, 184 rue du Faubourg Saint-Antoine, F-75012 Paris, France. Tel.: (1) 43.43.73.40. Telex: ARVIS 250 303 Public Paris.

**Chicago Area Medical Schools Obstetrics and Gynecology Review Course**, June 16-21, 1986, Chicago Hilton and Towers, Chicago, Illinois.

Contact: The University of Chicago, Office of Continuing Medical Education, 5841 Maryland, Box 139, Chicago, IL 60637. Tel.: (312) 962-1056.

**Reproductive Endocrinology and General Gynecology**, June 1-7, 1986, Sea Pines Plantation, Hilton Head Island, South Carolina. For further information contact: Jeanne Ryan, Program Coordinator, Office of Continuing Education, 720 Rutland Ave., Turner 22, Baltimore, MD 21205. Tel.: (301) 955-6046.

**Thirteenth Annual Symposium on Obstetrics and Gynecology**, April 24-25, 1986, Washington University Medical Center, St. Louis, Missouri. Contact: Loretta Giacometto, Washington University School of Medicine, Box 8063, 660 S. Euclid, St. Louis, MO 63110. Tel.: (800) 325-9862 outside Missouri; (314) 362-6893 in Missouri.

**Gynecologic Surgical Techniques**, June 12-14, 1986, Chicago, Illinois. For additional information, contact the Registrar's office. Toll-free number: Illinois 1 (800) 621-4649; outside Illinois 1 (800) 621-4651.

**Second Annual Long Island Assembly of OB/GYN—Crisis of the 80's: Medicolegal issues in OB/GYN**, May 29 and 30, 1986, Garden City Hotel, Garden City, New York. For information: Ann J. Boehme, Associate Director for Continuing Education, Long Island Jewish Medical Center, New Hyde Park, NY 11042. Tel.: (718) 470-8650.

**Basic and Advanced Laser Surgery in Gynecology,** July 31, August 1, and August 2, 1986, Sheraton Beach Inn, Virginia Beach, Virginia. For more information, please contact: Michael Baggish, M.D., Chief, Department of Ob/Gyn, Crouse Irving Memorial Hospital, 736 Irving Ave., Syracuse, NY 13210. Tel.: (315) 470-7903.

**Eighth Practical Course in Colposcopy and Cervico-Vaginal Histopathology (in English),** Paris, France, September 22-26, 1986. For further information contact: Dr. R. Cartier, 20 Rue Des Cordeliers, F75013 Paris, France.

**New Concepts in the Management of OB/GYN Infections,** September 3, 4, and 5, 1986, Grand

Hyatt, New York City. For further information contact: Selma Abdo, Continuing Education, Upstate Medical Center, 766 Irving Ave., Syracuse, NY 13210. Tel.: (315) 473-4304.

**Annual International Reproductive Health Seminar,** Quito, Ecuador, November 19-22, 1986. For further information contact: Howard A. Engle, M.D., Director General, IPARC, 975 41st St., Suite 102, Miami Beach FL 33140. Tel.: (305) 531-0047.

**Ultrasound 1986,** April 1-4, 1986. For more information contact: Department of Continuing Education, Harvard Medical School, 25 Shattuck St., Boston, MA 02115. Tel. (617) 732-1525.

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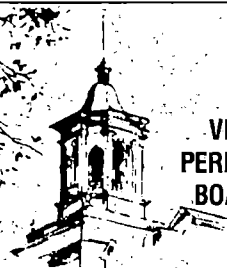
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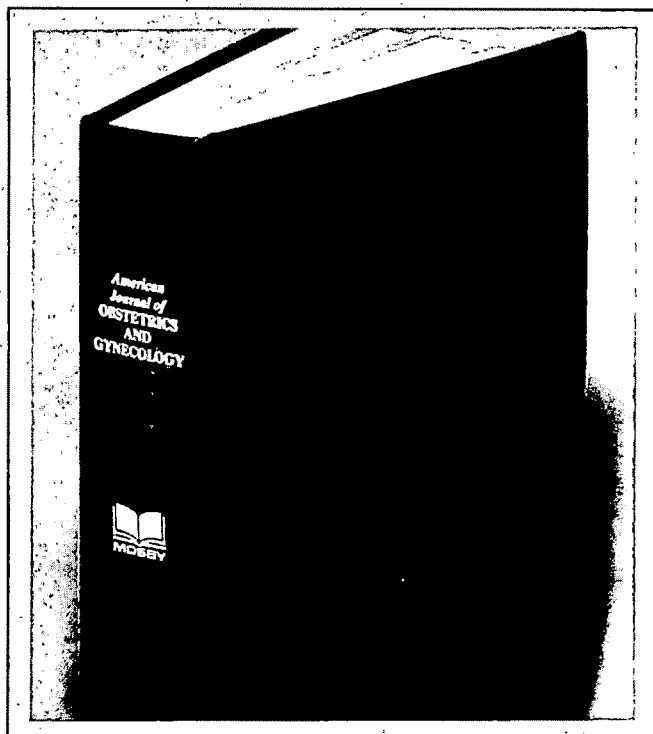
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## INDICATIONS AND USAGE: PREVENTION OF PREGNANCY.

**DOSE-RELATED RISK OF THROMBOEMBOLISM FROM ORAL CONTRACEPTIVES:** Two studies have shown a positive association between the dose of estrogens in oral contraceptives and the risk of thromboembolism. For this reason, it is prudent and in keeping with good principles of therapeutics to minimize exposure to estrogen. The oral contraceptive product prescribed for any given patient should be that product which contains the least amount of estrogen that is compatible with an acceptable pregnancy rate and patient acceptance. It is recommended that new acceptors of oral contraceptives be started on preparations containing 0.05 mg or less of estrogen.

**CONTRAINDICATIONS:** Oral contraceptives should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophlebitis or thromboembolic disorders. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Oral contraceptive tablets may cause fetal harm when administered to a pregnant woman. Oral contraceptive tablets are contraindicated in women who are pregnant. If the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus (see WARNINGS, No. 5). 8. Benign or malignant liver tumor which developed during the use of oral contraceptives or other estrogen-containing products.

## WARNINGS

**Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.**  
**The use of oral contraceptives is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.**

1. **THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS.** An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Four principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of oral contraceptive tablets are 4 to 11 times more likely than nonusers to develop these diseases without evident cause. Overall excess mortality due to pulmonary embolism or stroke is on the order of 1.0 to 3.5 deaths annually per 100,000 users and increases with age. **CEREBROVASCULAR DISORDERS:** In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than in nonusers and the risk of thrombotic stroke was 4.0 to 9.5 times greater in users than in nonusers. A prospective study conducted in Great Britain estimated that former users have a risk for all cerebrovascular disease 2.6 times greater than that of nonusers. This risk remained elevated for at least six years after last oral contraceptive use. A prospective study conducted in the United States found that past use of oral contraceptives was associated with increased risk of subarachnoid hemorrhage, the relative risk being 5.3. There was also some evidence from this study that the degree of risk may be related to duration of oral contraceptive use. **MYOCARDIAL INFARCTION:** An increased risk of myocardial infarction associated with the use of oral contraceptives has been reported confirming a previously suspected association. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preclampsia toxemia), the higher the risk of developing myocardial infarction, regardless of whether the patient was an oral contraceptive user or not. Oral contraceptives, however, were found to be a clear additional risk factor. The annual excess case rate (increased risk) of myocardial infarction (fatal and nonfatal) in oral contraceptive users was estimated to be approximately 7 cases per 100,000 women users in the 30-39 age group and 67 cases per 100,000 women users in the 40-44 age group. In terms of relative risk, it has been estimated that oral contraceptive users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. Oral contraceptive users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified. It is quite likely that the same synergistic action exists, but perhaps to a lesser extent. A study suggests that some increased risk of myocardial infarction in oral contraceptive users persists if following discontinuation of oral contraceptives and that the degree of the residual risk is related to the duration of the past use. **Risk of Dose:** In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in oral contraceptives. Preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the United States. Careful epidemiological studies to determine the degree of thromboembolic risk associated with progestogen-only oral contraceptives have not been performed. Cases of thromboembolic disease have been reported in women using these products, and they should not be presumed to be free of excess risk. The risk of thromboembolic and thrombotic disorders, in both users and nonusers of oral contraceptives, increases with age. Oral contraceptives are, however, an independent risk factor for these events. **ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES:** A large prospective study carried out in the United Kingdom estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of oral contraceptives according to age, smoking habits, and duration of use. The overall excess death rate annually from circulatory diseases for oral contraceptive users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), the risk being concentrated in older women, in those with a long duration of use, and in cigarette smokers. It was not possible, however, to examine the interrelationships of age, smoking, and duration of use, nor to compare the effects of continuous versus intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for five or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for five or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. This study reports that the increased risk of circulatory disease mortality may persist after the pill is discontinued. Another study published at the same time confirms a previously reported increase in mortality in patients from cardiovascular disease. The study concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of oral contraceptives in women over 40 who smoke. (The rates given for pill only/smokers for each age group are for smokers as a class. For "heavy" smokers [more than 15 cigarettes a day], the rates given would be about double; for "light" smokers [less than 15 cigarettes a day], about 50 percent.) The mortality associated with oral contraceptive use in nonsmokers over 40 is higher than with any other method of contraception in that age group. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with oral contraceptives increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of preclampsia toxemia and especially by cigarette smoking. The risk of myocardial infarction in oral contraceptive users is substantially increased in women age 40 and over, especially those with other risk factors. The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four- to six-fold increased risk of postsurgery thromboembolic complications has been reported in oral contraceptive users. If feasible, oral contraceptives should be discontinued at least four weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization. 2. **OCULAR LESIONS:** There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of oral contraceptives. Discontinue oral contraceptive medication if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia, papilledema, or retinal vascular lesions and institute appropriate diagnostic and therapeutic measures. 3. **CARCINOMA:** Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in oral contraceptives, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case control studies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on oral contraceptives. Of the cases found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time oral contraceptives were first given, polycystic ovaries), nearly all occurred in women who had used a sequential oral contraceptive. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only oral contraceptives. Several studies have found no increase in breast cancer in women taking oral contraceptives or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with oral contraceptives, found an excess risk in the subgroups of oral contraceptive users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of oral contraceptives has been well-documented. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with oral contraceptives. Close clinical surveillance of all women taking oral contraceptives is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. 4. **HEPATIC TUMORS:** Benign hepatic adenomas have been found to be associated with the use of oral contraceptives. One study showed that oral

contraceptive formulations with high hormonal potency were associated with a higher risk than lower potency formulations and use of oral contraceptives with high hormonal potency and age over 30 years may further increase the woman's risk of hepatocellular adenoma. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of oral contraceptives. Two studies relate risk with duration of use of the contraceptive, the risk being much greater after four or more years of oral contraceptive use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women taking oral contraceptives. The relationship of these drugs to this type of malignancy is not known at this time. 5. **USE IN OR IMMEDIATELY PRECEDING PREGNANCY, BIRTH DEFECTS IN OFFSPRING, AND MALIGNANCY IN FEMALE OFFSPRING:** The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be on the order of 1 to 4 in 1000 exposures. Although there is no evidence at the present time that oral contraceptives further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including oral contraceptives, in pregnancy. One case control study has estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing oral contraceptives. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping oral contraceptives is unknown. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period (or after 45 days from the last menstrual period if the progestogen-only oral contraceptives are used), and further use of oral contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue oral contraceptives with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend three months although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy. 6. **GALLBLADDER DISEASE:** Studies report an increased risk of surgically confirmed gallbladder disease in users of oral contraceptives and estrogens. In one study, an increased risk appeared after two years of use and doubled after four or five years of use. In one of the other studies, an increased risk was apparent between six and twelve months of use. 7. **CARBOHYDRATE AND LIPID METABOLIC EFFECTS:** A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. For this reason, prediabetic and diabetic patients should be carefully observed while receiving oral contraceptives. An increase in triglycerides and total phospholipids has been observed in patients receiving oral contraceptives. The clinical significance of this finding remains to be defined. 8. **ELEVATED BLOOD PRESSURE:** An increase in blood pressure has been reported in patients receiving oral contraceptives. In some women hypertension may occur within a few months of beginning oral contraceptive use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence increases, however, with longer exposure, and in the fifth year of use is two and a half times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in oral contraceptive users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given oral contraceptives. Hypertension that develops as a result of taking oral contraceptives usually returns to normal after discontinuing the drug. 9. **HEADACHE:** The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of oral contraceptives and evaluation of the cause. 10. **BLEEDING IRREGULARITIES:** Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an oral contraceptive with higher estrogen content, while potentially useful in minor menstrual irregularities, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of oral contraceptives. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Postuse anovulation, possibly prolonged, may also occur in women without previous irregularities. 11. **ECTOPIC PREGNANCY:** Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. 12. **BREAST FEEDING:** Oral contraceptives given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of oral contraceptives should be deferred until the infant has been weaned. **PRECAUTIONS: General:** 1. A complete medical and family history should be taken prior to the initiation of oral contraceptives. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, oral contraceptives should not be prescribed for more than one year without another physical examination being performed. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking oral contraceptives should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. 4. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving oral contraceptive therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. Oral contraceptive users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by oral contraceptive therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping oral contraceptives, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of oral contraceptive therapy when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing oral contraceptives: a. Increased sulfobromophthalen retention; b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin; c. increased norepinephrine-induced platelet aggregability; d. Increased thyroid-binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered; d. Decreased pregnanediol excretion; e. Reduced response to metyrapone test. **INFORMATION FOR THE PATIENT: (See Patient Package Insert).** **DRUG INTERACTIONS:** Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin, griseofulvin, and tetracycline. **CARCINOGENESIS, PREGNANCY, NURSING MOTHERS: See CONTRAINDICATIONS AND WARNINGS. ADVERSE REACTIONS:** An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS): Thrombophlebitis. Pulmonary embolism. Coronary thrombosis. Cerebral thrombosis. Cerebral hemorrhage. Hypertension. Gallbladder disease. Liver tumors. Congenital anomalies. There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis. Neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea, usually the most common adverse reaction. Vomiting, occurs in approximately 10% or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Dysmenorrhea. Amenorrhea during and after treatment. Temporary infertility after discontinuance of treatment. Edema. Chloasma or melasma which may persist. Breast changes: tenderness, enlargement, and secretion. Change in weight (increase or decrease). Change in cervical erosion and cervical secretion. Possible diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine. Increase in size of uterine leiomyomata. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives, and the association has been neither confirmed nor refuted: Premenstrual-like syndrome. Cataracts. Changes in libido. Chorea. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiforme. Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. Hemolytic uremic syndrome. **OVERDOSAGE:** Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

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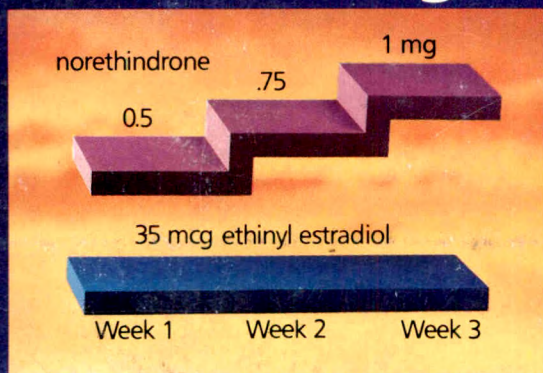


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†Serious as well as minor side effects have been reported with the use of oral contraceptives. The physician should remain alert to the earliest manifestations of any symptoms of serious disease and discontinue oral contraceptive therapy when appropriate. Please see complete Prescribing Information, a summary of which appears on the preceding page.

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